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HISTOPATHOLOGY

by

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16. ABSTRACT

Overt signs of toxicity (loss of appetite, loss of equilibrium, discoloration, or death) are nearly always preceded by biochemical, physiological, and/or morphological changes in the organism. The ability to qualitatively or quantitatively measure these changes prior to death of the organism can often provide early indications of toxicity and valuable insights into the mechanisms of toxicity. This chapter concerns the field of histopathology and its importance, use, and application in aquatic toxicology. Histopathology is the study of the structure of abnormal tissue. Examination of tissues from fish and other aquatic organisms after death may serve to identify the cause of death and possibly the causative agent. This information along with physiological and biochemical data may provide a more complete and accurate description of the activity of a chemical agent.

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Histopathology

T. R. Meyers and J. D. Hendricks

INTRODUCTION

Overt signs of toxicity (loss of appetite, loss of equilibrium, discoloration, or death) are nearly always preceded by biochemical, physiological, and/or morphological changes in the organism. The ability to qualitatively or quantitatively measure these changes prior to death of the organism can often provide early indications of toxicity and valuable insights into the mechanisms of toxicity. This chapter concerns the field of histopathology and its importance, use, and application in aquatic toxicology. Histopathology is the study of the structure of abnormal tissue. Examination of tissues from fish and other aquatic organisms after death may serve to identify the cause of death and possibly the causative agent. This information along with physiological and biochemical data may provide a more complete and accurate description of the activity of a chemical agent.

Although major advances have been made in recent years, the histology and histopathology of fish and aquatic invertebrates are still infant sciences compared with counterparts in mammals. There is probably no aquatic species whose normal histology is completely known. Only four atlases of normal histology exist: for the rainbow trout (Anderson and Mitchum, 1974), the channel catfish (Grizzle and Rogers, 1976), the American oyster (Galtsoff, 1964), and the blue crab (John-

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son, 1980). These have been important contributions to the field, but represent only a fraction of the comprehensive literature that is available for mammals. The individual who desires to work in the area of aquatic animal histology or histopathology must draw heavily from basic mammalian histological and pathological concepts that are applicable to all living tissues and from the scattered literature on aquatic animal histology and pathology. Ultimately, such an individual may become the expert on a particular aquatic species, since it is possible that no one else has studied it.

The following sections present some routine diagnostic methods that may be used in aquatic histopathology to evaluate abnormal tissue changes resulting from exposure of the organism to a toxicant. Also included are definitions of commonly used terms and a review of the literature on toxic histopathology in aquatic organisms.

TERMINOLOGY

A glossary is presented below to define certain pathology terms mentioned within the text and literature review. Most definitions have been adapted from Stedman's Medical Dictionary (1976), Smith et al. (1972), and Schalm (1970). Another useful reference is the Glossary of Fish Health Terms (Post and Klontz, 1977).

Adenoma. Benign epithelial tumor in which cells form fairly well-differentiated glandular structures.

Adenomatous. Resembling an adenoma, sometimes referring to glandular hyperplasia.

Anemia. Reduction in the oxygen transport capabilities per unit volume of blood due to below-normal numbers of erythrocytes and/or a decreased hemoglobin concentration. Anemias are usually secondary diseases and are classified by cause and on the basis of morphological characteristics of erythrocytes.

Anisochromasia. Variability in staining characteristics of individual cell cytoplasms and/or nuclei within a population of cells; unequal cytoplasmic staining within a single cell as seen in erythrocytes from certain anemias due to unequal distribution of hemoglobin.

Anisocytosis. Extreme differences in cell size within a normally uniform population, particularly seen in certain blood diseases.

Anophthalmia. Congenital defect where one or both eyes are absent.

Atrophy. Wasting of cells, organs, or entire body resulting from various causes including necrosis and reabsorption of cells, decreased cellular proliferation, malnutrition, decreased use, pressure, ischemia, or hormonal changes.

Ballooning degeneration. Separation of epithelial cells from one another classically seen in the deeper cell layers of the epidermis. Usually follows intracytoplasmic edema and vacuolation.

Catarrhal enteritis. Inflammation of any or all of the intestinal tract characterized by excessive mucus production accompanied by epithelial necrosis and desquamation. Necrosis may often predominate, in which case little or no mucus is evident from lack of a secreting membrane.

Centrilobular. Refers to the central area of hepatic parenchyma immediately surrounding the central vein of a liver lobule.

Ceroid. High molecular weight intracellular lipofuscin pigment resulting from autoxidation of phospholipids and unsaturated fats accompanied by polymerization. Thus, ceroid is insoluble in organic solvents, characteristically acid-fast, variably PAS-positive, sudanophilic in both frozen and paraffin sections, and negative with iron stains. Ceroid is found in cases of vitamin E deficiency, wasting diseases, and old age (Figs. 1 and 2).

Cirrhosis. Proliferative form of chronic hepatic inflammation characterized by replacement of parenchyma by new fibrous connective tissue generally beginning at the portal triads.

Cloudy swelling (acute cellular swelling). Earliest degenerative change within a cell, caused by influx of water, characterized by enlarged cell and homogeneous "ground glass" appearance of cytoplasm (Fig. 3).

Congestion. Excessive amount of blood within vessels producing a "pavementing" effect in the

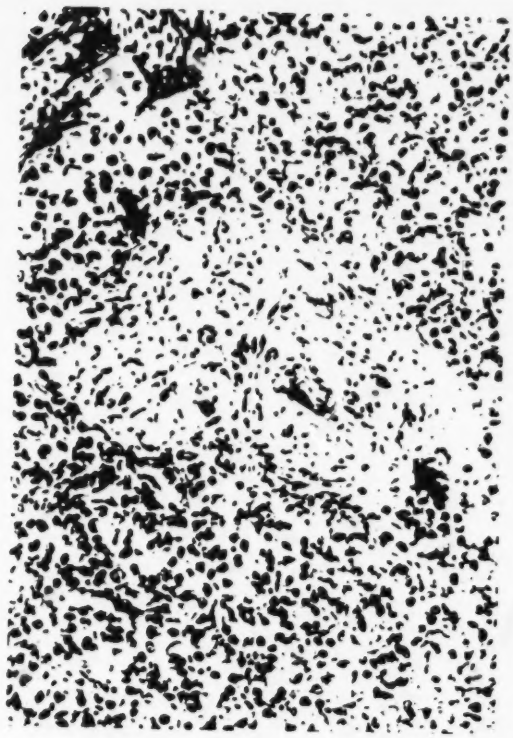

Figure 1 Portion of liver from a yearling rainbow trout after 1 yr on a diet containing 50 ppm sterculic acid, a cyclopropenoid fatty acid. Note the clear, lightly staining material in the cells of the expanded melanomacrophage center (arrows). H&E, ×440.

lumina. Cause is usually insufficient venous drainage of blood (passive).

Degenerative changes. Early abnormal prenecrotic changes in cellular detail, many of which are reversible up to a certain point (i.e., fatty change, hydropic degeneration, cloudy swelling, etc.).

Desquamation. Sloughing of cells from an epithelial surface due to necrosis, some degenerative change, or from postmortem autolysis (Fig. 4).

Dysplasia. Abnormal alteration in size, shape and organization of adult cells or tissue (Figs. 5 and 6).

Edema. Excessive amounts of water collecting in intercellular spaces and/or body cavities.

Electron microscopy (EM). Use of a high-voltage electron beam with shorter wavelengths than visible light to observe fine structural detail not resolved with conventional light microscopy. Two-dimensional (transmission EM) and three-dimensional (scanning EM) viewing are routinely used.

Epithelioma. Benign neoplasm involving epithelial cells of the epidermis, including those of the adnexa.

Figure 2 Frozen section of liver from the fish shown in Fig. 1, but stained with Sudan Black. Melanomacrophage centers are intensely sudanophilic, indicating the presence of a lipid material. In paraffin sections this material was also positive with periodic acid-Schiff reagent and was acid-fast, both characteristics of a ceroid pigment. ×440.

Figure 3 Section of liver from a 9-mo-old rainbow trout fed the control diet plus 100 ppm pyrrolizidine alkaloids for 6 mo. Replacement of degenerate hypertrophied hepatocytes with extensive fibrosis (f), cloudy swelling (c) of hepatocytes, and bile ductule hyperplasia (h) are illustrated. H&E, ×440. *[From Hendricks et al. (1981).]*

Erythema. Redness of the skin caused by inflammation.

Exophthalmos ("popeye"). Unilateral or bilateral extrusion of the eyes from their sockets commonly caused by accumulation of gas bubbles (nitrogen "gas bubble" disease) or edema fluids (osmoregulatory problems due to kidney lesions, etc.) beneath the eye globes.

Extravasation. Passing outside the vasculature, usually referring to blood or lymph. A large hemorrhage within a tissue.

Fatty change. Intracytoplasmic accumulation of neutral fat droplets. It is considered to be a degenerative change independent of, but often occurring with, necrosis and is commonly found in the liver and kidney epithelium (Figs. 7 and 8). Fatty change results from inhibition of transport or metabolism of lipid. Because neutral fat is dissolved by organic solvents used in histological preparation, it appears as well demarcated unstained areas within the cell cytoplasm.

Fibrinoid degeneration. Degeneration occurring in fibrous connective tissue, probably resulting from a local antigen-antibody reaction. It appears to be either fibrin from an inflammatory exudate or degenerative collagen, which stains dirty pink

Figure 4 Portions of two gill filaments from a yearling coho salmon exposed to the herbicide dinoseb at 100 ppm in water for 114 h. Note the nearly complete necrosis and desquamation of the respiratory epithelium from the underlying pillar cells and blood sinuses. H&E, ×440.

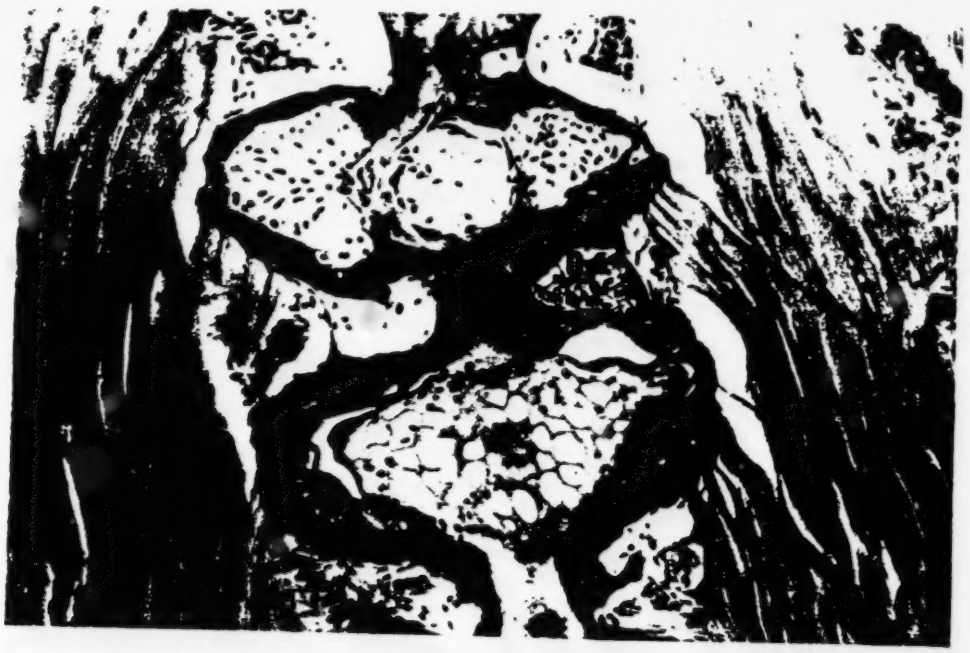

Figure 5 Vertebral dysplasia in *Cyprinodon variegatus* exposed for 30 d to 31 µg/l trifluralin in flowing seawater. Note the enlarged notochord (n), persistence and proliferation of osteoblasts (o) and/or fibroblasts (f), and semisymmetric hypertrophy (h) of the vertebral walls. H&E, ×258. *[Courtesy of Dr. John Couch, U.S. Environmental Protection Agency, Gulf Breeze, Florida.]*

and has fewer nuclei than surrounding normal tissues.

Fibroplasia or fibrosis. Adequate or excessive production of new fibrous connective tissue as a repair response to cell death following or concomitant with inflammation (Fig. 3).

Granuloma. Mass or focus of granulation tissue. There are two basic types: fibrous granuloma, which is composed of inflammatory cells, actively proliferating fibrous connective tissue, and numerous capillary buds, and reticuloendothelial granuloma, which contains proliferating macrophages or histiocytes, usually circumscribed by a collar of lymphocytes. A granuloma is a chronic host defense mechanism to wall off the site of infectious, toxic, or mechanical irritation.

Heinze bodies. Round, oval to serrated, cytoplasmic inclusion bodies observed within erythrocytes, usually indicative of hemolytic anemia following exposure to certain toxic compounds. Inclusions are refractile when unstained, blue when stained with new methylene blue. They are usually located adjacent to or protruding from the cell wall, but can be found free in the blood plasma.

Hemorrhage. Presence of blood outside the vasculature due to injury of the vessel walls.

Hemostasis. Stoppage of blood flow either by hemorrhage or in part of the vascular circulation.

Histochemistry. Qualitative identification of the normal and abnormal intracytoplasmic distribution of various cell products and assimilated chemicals (lipids, polysaccharides, nucleic acids, enzymes, etc.) and reaction sites by use of several methods including specific chemical stains, immunofluorescence, autoradiography, and electron microscopy.

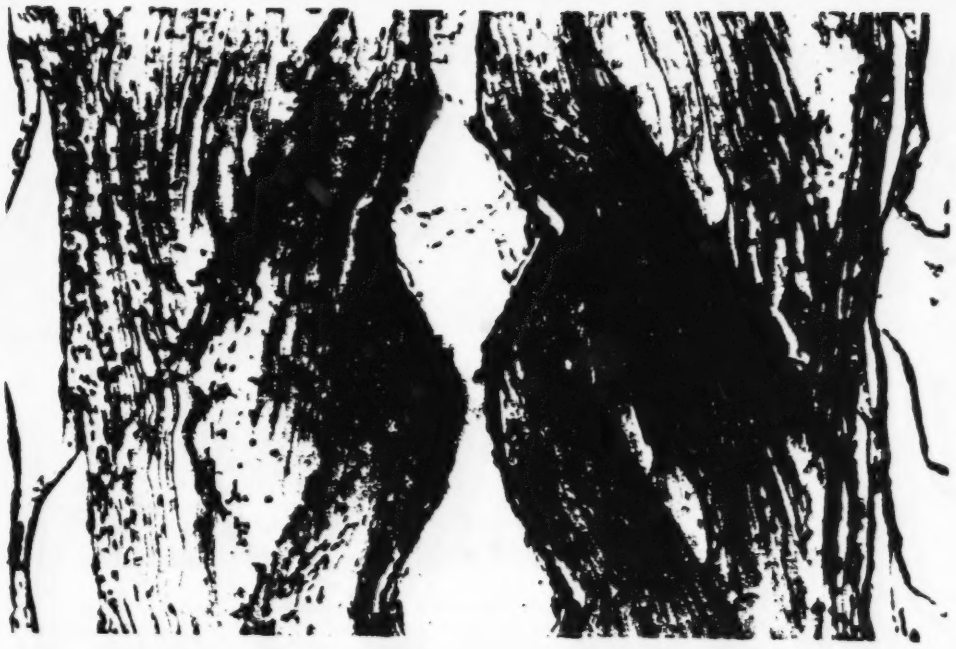

Figure 6 Normal vertebral structure from a control *Cyprinodon variegatus*, sectioned at the same level as the trifluralin-exposed specimen. Note vestigial notochord and thin-walled vertebrae. H&E, ×258. *[Courtesy of Dr. John Couch.]*

Histology. Study of normal microscopic and ultrastructural anatomy of animals or plants including the structure and function of cells, tissues, and organs. This is done by observing stained paraffin tissue sections with light microscopy and ultrathin sections with electron microscopy.

Howell-Jolly bodies. Round nuclear remnants eccentrically located in a young erythrocyte, usually one per cell. Can be indicative of a remissive anemia, but occur normally at low levels in the cat and the horse.

Hyalin degeneration. Cellular change characterized by glassy homogeneous eosinophilic material seen as droplets within cell cytoplasm or found replacing entire cells or cellular areas.

Hydropic degeneration. Accumulation of water within the cytoplasm of a swollen epithelial cell, appearing as a clear vacuole or vacuoles with an indistinct edge often adjacent to or surrounding the nucleus. This change may be a further sequence of cloudy swelling (Fig. 9).

Hyperchromic. More intensely staining.

Hyperemia. Excessive blood within the vasculature, distinguished from congestion by its "active" cause—dilated arteries delivering too much blood for proper venous drainage.

Hyperplasia. Increased proliferation of a cell population, usually in response to an irritant (toxic or infectious), endocrine imbalance, or nutritional deficiency. Normal tissue function and architecture may be impaired as observed in gill epithelium when hyperplasia causes overgrowth of cells ("clubbing") and cellular fusion of filaments and lamellae (Figs. 3, 10, and 11).

Hypertrophy. Increase in individual cell size and functional ability, sometimes resulting in a

concomitant increase in tissue or organ size. Hypertrophy can occur from an increase in functional demand, which may be compensatory or hormonal in origin (Figs. 3, 5, 6, 7, 8, 10, 11).

Infarct. Localized area of tissue necrosis (usually coagulative) caused by insufficient blood supply due to vascular obstruction.

Inflammation. Dynamic process of events involving vascular and exudative stages occurring only in viable tissues that have been injured by an irritant. This response permits humoral and cellular elements of the immune system to interact with the causative agent at local sites of tissue damage in order that healing may occur. Inflammation is classified according to the morphological nature of the exudate, which varies with the type of tissue involved and the causative agent. Six forms of inflammation are recognized:

(*a*) *Serous inflammation*, characterized by a watery exudation of blood serum, often in peritoneal cavities or formed vesicles, with microscopic appearance of homogeneous pink staining precipitate mixed with a few leukocytes and slight amounts of fibrin.

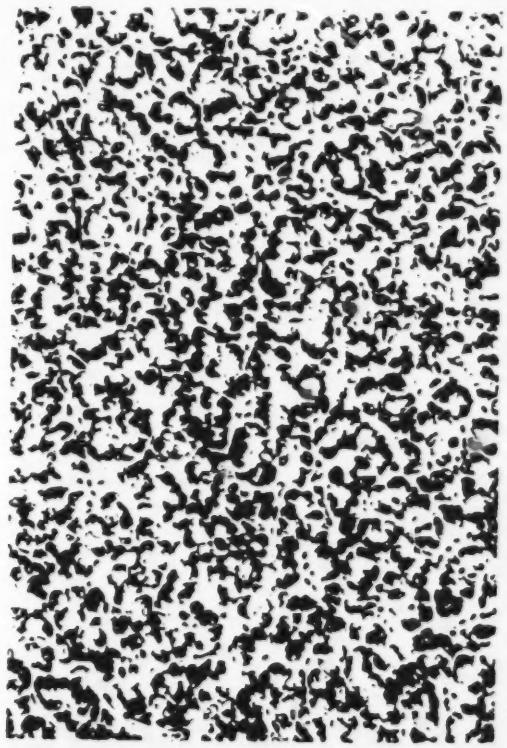

Figure 7 Portion of normal liver from 9-mo-old control rainbow trout. Note the small uniform size of the hepatic parenchymal cells and their nuclei. Hepatic tubulocords are two cells in width. H&E, ×440. *[From Hendricks et al. (1981).]*

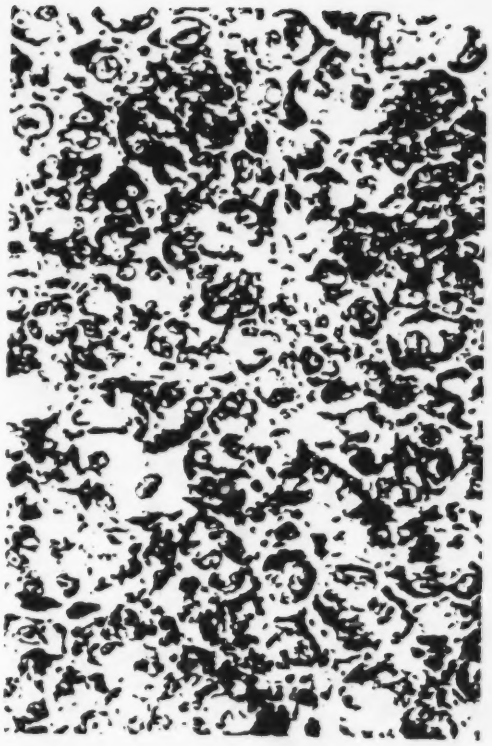

Figure 8 Portion of liver from a 9-mo-old rainbow trout fed the control diet plus 20 ppm mixed pyrrolizidine alkaloids from tansy ragwort plant for 6 mo. Note the great hypertrophy (megalocytosis) of the hepatocytes, the microdroplet fatty change (double arrow) in some of the cells, the abnormal cell shapes and staining characteristics, and intranuclear inclusion bodies (single arrow). Two foci (f) of small basophilic, regenerating hepatocytes are visible in the upper portion of the photomicrograph. H&E, ×440. *[From Hendricks et al. (1981).]*

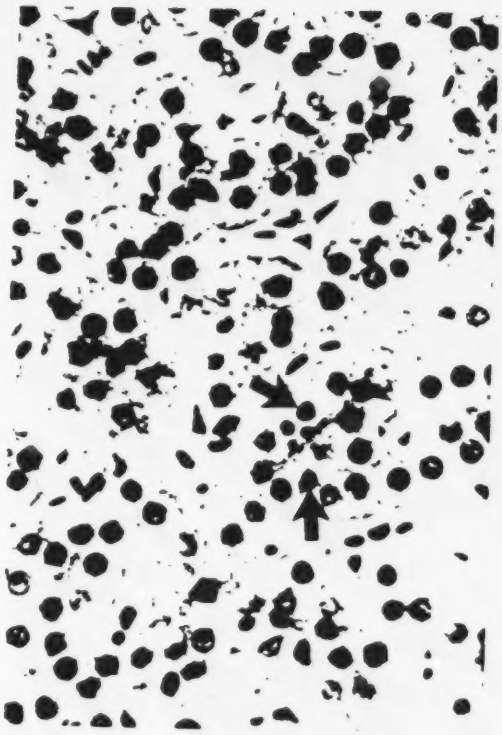

Figure 9 Portion of liver from a yearling coho salmon water exposed to 100 ppm of the herbicide amitrole for 144 h. Note the extreme hydropic degenerative changes in the hepatocytes, visualized as clear cytoplasmic vacuoles, some surrounding the nuclei. Several cells in each hepatic cord have undergone necrosis, as evidenced by pyknotic nuclei (arrows). H&E, X1101.

(*b*) *Fibrinous inflammation*, with an exudate composed predominantly of clotted fibrin, usually occurring on mucous and serous membranes, particularly the pericardium. Microscopically, the exudate contains dirty pink staining fibrillar material adhering to a hyperemic parent surface usually accompanied by variable amounts of leukocytes, erythrocytes, and other precipitated serum proteins. A thick fibrin exudate that becomes firmly attached to its parent surface denotes a diphtheritic inflammation.

(*c*) *Purulent inflammation*, in which the exudate contains pus, which consists of variable numbers of necrotic and living neutrophils in or on the tissues accompanied by congestion and small amounts of fibrin, serum, and other leukocytes. If neutrophils predominate over other elements, the inflammation is said to be "suppurative." Although fish have neutrophils, purulent inflammation, particularly abscesses (circumscribed accumulations of pus), rarely occurs in teleosts.

(*d*) *Hemorrhagic inflammation*, consisting of an exudate that may contain many of the other exudative components above but also large accumulations of erythrocytes obviously outside vascular channels. This exudate can occur diffusely

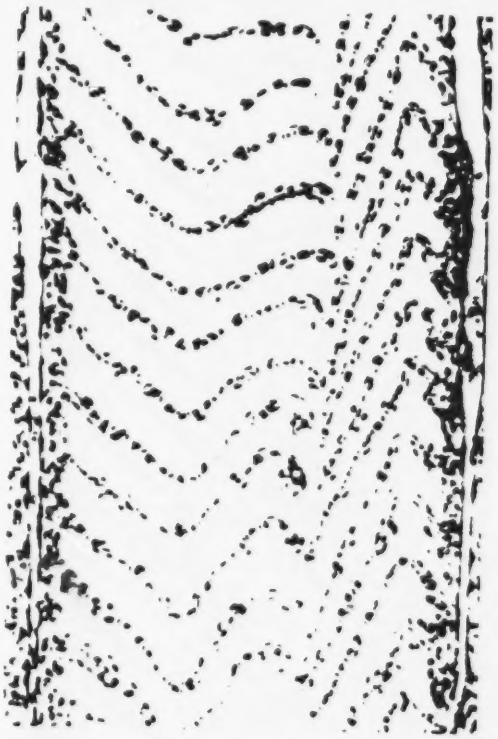

Figure 10 Normal gill structure from a yearling control coho salmon (*Oncorhynchus kisutch*). Note the single layer of squamous epithelial cells covering the lamellae and the basal interlamellar regions of the gill filament. H&E, x440.

within tissues but more often involves mucous membranes.

(*e*) *Catarrhal or mucous inflammation*, which involves only mucous membranes since the principal component of the exudate is mucous secreted from host cells. Under the microscope, affected membranes may demonstrate hyperplasia of mucous goblet cells accompanied by attached overlying gray- or blue-staining strands of mucin. More often the membrane surface undergoes necrosis and desquamation, exposing an underlying hyperemic connective tissue infiltrated with varying amounts of lymphocytes.

(*f*) *Lymphocytic inflammation*, which results from infiltration of lymphocytes within affected tissues accompanied by some hyperemia. Histologically, tissues appear normal, except for slight vascular congestion and collections of lymphocytes, often surrounding the vessels (perivascular cuffing). Tissues in which this type of inflammation commonly occurs include nervous tissue, portal triads of the liver, and lamina propria of the gut. The latter two areas normally have resident populations of lymphocytes, which vary in number according to animal species.

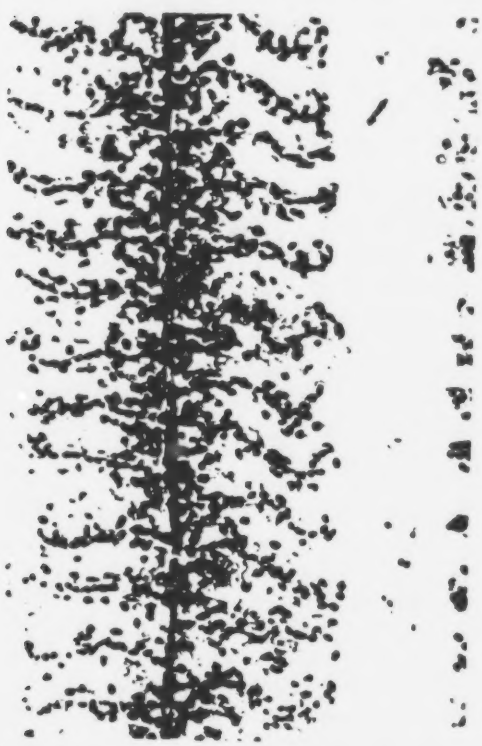

Figure 11 Gill filament from a yearling coho salmon following water exposure to 100 ppm paraquat for 120 h. Note both hypertrophy of individual epithelial cells and hyperplasia, particularly in the basal interlamellar region. H&E, X440.

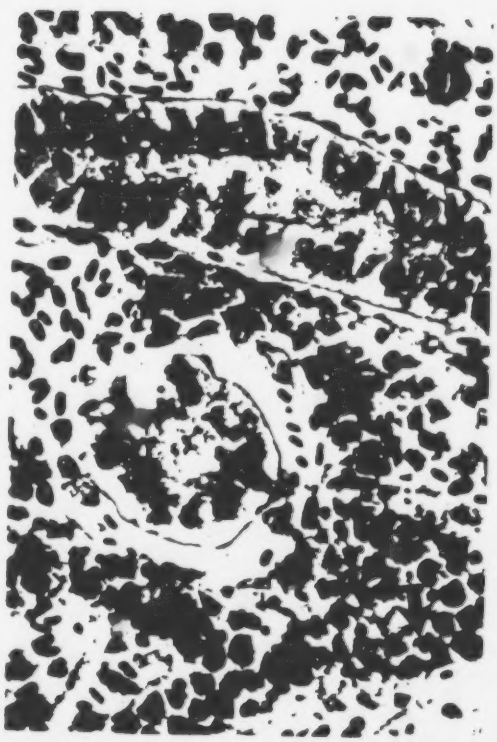

Figure 12 Portion of kidney tissue from a yearling coho salmon following water exposure to 100 ppm of the herbicide amitrole for 144 h. All the tubular cells and many of the surrounding hematopoietic cells have undergone necrosis; nuclear changes of pyknosis and karyorrhexis (arrows) are clearly visible. H&E, X1101.

Ischemia. Insufficient blood supply.

Karyolysis. Necrotic change characterized by

dissolution of a cell nucleus, recognized by incomplete stages where only nuclear outlines are visible.

Karyorrhexis. Necrotic change characterized by rupture of the nuclear membrane and fragmentation of the cell nucleus. These fragments may remain at the original site of the nucleus or may be scattered about with other necrotic debris (Fig. 12).

Lesion. Abnormal change within a cell, tissue, or organ.

Leukopenia. Abnormal reduction in circulating white blood cells per unit volume of blood.

Metaplasia. Change from one cell type to another within the limits of the original primary tissue, such as squamous cell metaplasia of cuboidal or columnar epithelium. Metaplasia has been seen only in epithelium and connective tissue. The cause is a change in demand of function.

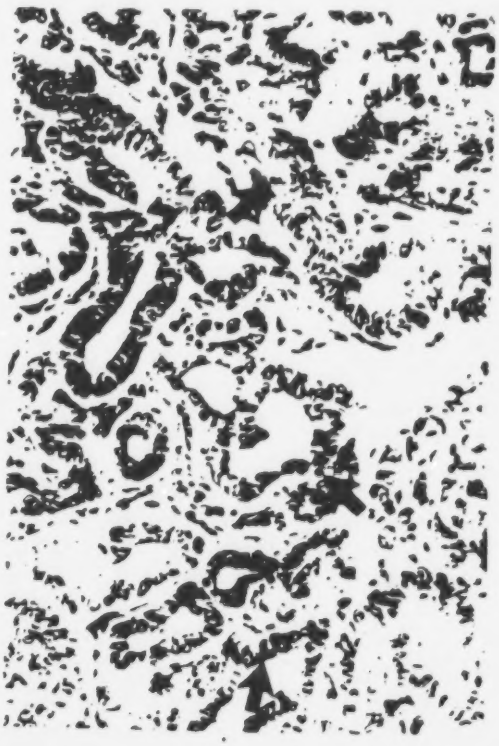

Figure 13 Portion of a cholangiocarcinoma induced in the liver of a rainbow trout by continuous feeding of 25 ppm *p,p'*-DDT for 21 mo. Note the irregular nature of the bile ducts and the frequent mitotic figures (arrows). H&E, ×440.

Microphthalmia. Congenital defect in which one or both eyeballs are abnormally small.

Mucin. Mucopolysaccharide cellular secretion that stains gray to blue with hematoxylin and eosin.

Mucus. Viscid complex cellular secretion that functions as a cleansing agent, lubricant, and/or protective barrier at various membrane surfaces. Mucus is comprised of mucin, epithelial cells, leukocytes, and various inorganic salts dissolved in water, and may contain immunoglobulins and lysozymes, which provide localized protection against infectious agents.

Myositis. Inflammation of smooth or skeletal muscle.

Myxomatous degeneration (mucoid degeneration; serous atrophy of fat). Proliferation of and replacement by a primitive embryonal-type connective tissue where fibrous and/or adipose connective tissues occur. Myxomatous tissue is composed of a mucinous grayish to blue loosely arranged fibrillar ground substance containing fibroblastic cells with stellate to spherical hyperchromatic nuclei. The primary cause is poor nutrition, although certain toxemias may play a role.

Necrosis. Cell or tissue death within a living body. Necrosis is classified as several histological types, including:

(*a*) *Coagulative necrosis*, death of cells in which nuclei become pyknotic, cytoplasm becomes acidophilic, and cellular outlines and other tissue structures are still visible (Figs. 4, 9, 12).

(*b*) *Caseous necrosis*, loss of differential staining with disintegration of tissue structures and cell membranes into a homogeneous mixture of granular debris that is basophilic to purple with hematoxylin and eosin.

(*c*) *Liquefactive necrosis*, rapid and complete dissolution of cells and tissue structures leaving

ragged-edged spaces filled with fluid, which may or may not have a residue of pink proteinaceous material when stained. An abscess produces a form of liquefactive necrosis in which fluid contains neutrophils, fibrin, and cellular debris.

(*d*) *Fat necrosis*, saponification of neutral fat into solvent-stable soap appearing solid and opaque within the dead fat cells. The soap may have small clefts from dissolved fatty acid crystals and should stain pink, purple, or blue, depending on which metallic ions (potassium, calcium, sodium, respectively) combined with the fatty acid radical during soap formation.

(*e*) *Zenker's necrosis*, which is similar to coagulative necrosis but occurs only in skeletal muscle, where changes are characterized by swelling of individual muscle fibers, marked homogeneous texture and acidophilia of the sarcoplasm, obscurity of myofibrils (striations), and pyknosis of nuclei.

Further descriptions of necroses may be based on distribution or location of the lesion—diffuse, focal, perivascular, and so on.

Neoplasia. Pathological transformation of a normal host cell resulting in uncontrolled proliferation into new cells without normal histological architecture or normal cellular functions. These cells usually bear some resemblance to the normal tissue of origin, although some malignant forms may appear so undifferentiated that cell identification of the growth or tumor is difficult (Fig. 13). For a discussion of tumor classification see the section on general types of tissue changes.

Nephrosis. Degenerative changes that may lead to necrosis in the nephrons of the kidney which do not involve an inflammatory process (called nephritis). Blood-borne toxic materials are common causes of nephrosis, particularly in renal tubular epithelium (Fig. 12).

Neutrophilia. Abnormally high percentage of neutrophil granulocytes in circulating blood, which may also cause an absolute increase in total number of peripheral white blood cells (leukocytosis).

Osteoblast. The cell responsible for the formation of osteoid or bone matrix. An osteoblast is eventually surrounded by bone and then becomes an osteocyte (Fig. 5).

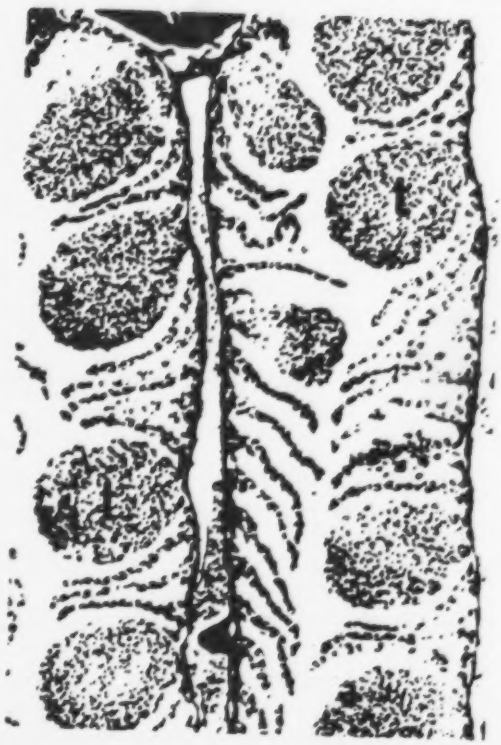

Figure 14 Portions of two gill filaments from a yearling coho salmon exposed to the herbicide atrazine at 15 ppm in water for 140 h. Note the severe telangiectasia (t) of the blood sinuses of the individual lamellae. H&E, X275.

Pathology. The medical science concerned with the study of abnormal changes, and their causes, within the cells, tissues, and organs of an individual or a population.

Periportal. Referring to the area of tissue immediately surrounding a portal triad within the liver.

Petechiae. Small pinpoint hemorrhages.

Poikilocytosis. Distortion or abnormal variation in cell shape, usually referring to erythrocytes in certain blood diseases.

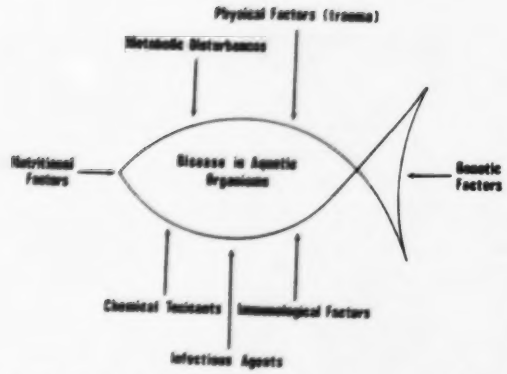

Figure 15 Etiologic factors of disease in aquatic organisms.

Polycythemia (erythrocytosis). Abnormal increase in the number of red blood cells per unit volume of blood.

Proliferative glomerulonephritis. Inflammation of the kidney glomeruli characterized by hypertrophy of glomerular tufts due to increased infiltration of leukocytes and proliferation of endothelial and visceral epithelial cells (podocytes). The increased cellularity causes compression of glomerular capillaries, leading to thrombosis and necrosis with subsequent hemorrhage into Bowman's space. Proliferating podocytes may cause adhesions with the parietal epithelium of Bowman's capsule, producing "epithelial crescents." There is no thickening of the glomerular basement membrane or alteration in foot processes of podocytes; these 2 anomalies help distinguish this condition from membranous glomerulonephritis.

Pyknosis. Necrotic change in cell nuclei characterized by a rounding up and condensation that causes hyperchromatic staining (Figs. 9 and 12).

Scoliosis. Lateral curvature of the spinal column. A combination of backward curvature with the lateral curvature is termed lordoscoliosis.

Telangiectasia. Marked dilation of terminal blood vessels within a part, usually capillaries (Fig. 14).

Thrombocytopenia. Abnormal reduction in thrombocyte or platelet cell numbers per unit volume of blood.

Thrombus. Blood clot, formed within a vessel or heart chamber of a living animal, which may obstruct blood flow or only partly so by attaching to the vessel wall.

Vasodilation. Enlargement of a vessel lumen.

POSSIBLE ETIOLOGIES OF HISTOPATHOLOGY IN AQUATIC ORGANISMS

In the science of pathology, the investigator must first know the normal variations of gross and microscopic anatomy in the necropsy (autopsy) subject. This is a challenge with fish and shellfish because there are many species that often differ somewhat in gross and histological appearance. For example, in lake trout the epithelium of the small distal portion of the convoluted tubule in the kidney often contains large cytoplasmic vacuoles (Walsh and Ribelin, 1975). In other fish species this might suggest a degenerative change, possibly resulting from toxic materials in the glomerular filtrate. In experimental situations, the use of normal unexposed control animals maintained under identical environmental conditions should eliminate confusion of normal features with those of true lesions. Although some field studies may require pathological interpretations without adequate control tissues for comparison, it cannot be overstressed that controls are indispensable for diagnosis of specific lesions and their etiologies.

The investigator must also be able to recognize postmortem pathology that begins immediately after death. This is due to the autolytic processes of extracellular and intracellular enzymes and to putrefaction by resident bacterial flora. Consequently, accurate interpretation of cellular and tissue morphology requires preservation and examination of tissues as soon after death as possible. For a useful discussion of invertebrate postmortem changes, the reader is referred to Sparks (1972).

Equally important is the distinction of toxi-

cological lesions from those that may have resulted from other causes (Fig. 15). Collection, handling, and sampling procedures and design of animal maintenance facilities should be examined as possible sources of traumatic or self-inflicted injuries. Fin nipping by other fish and tank abrasions are quite common; they often result in white necrotic areas at fin tips or scraped and ulcerated regions on the body surface, particularly on the snout. These lesions may become secondarily infected with bacterial or fungal organisms and may ultimately lead to morbidity. In wild fish predator attacks can lead to stab, bite, or scrape lesions, which eventually heal via granulation tissue unless also infected by an opportunistic pathogen. Other physical factors in the environment can cause lesions in fish: particulate irritants including silt, ash, and dust can abrade delicate gill tissues, which usually respond by proliferation (hyperplasia) of epithelium; supersaturation of dissolved gases (nitrogen, oxygen) in the water can cause formation of gas bubbles in fish tissues (gas bubble disease). Lesions resulting from gas bubble disease may include external swellings, exophthalmia, gas emboli in vasculature, and edema of secondary gill lamellae and other tissues as well. Sunburn, probably caused by excessive exposure to ultraviolet radiation, can occur in cultured fish kept in uncovered shallow raceways, but it has also been reported in feral fish. The typical lesion is a focal necrosis on the crown of the head, which may become secondarily infected with bacteria or fungi. In young fish, necrosis and hemorrhage may involve the meninx primitiva and optic lobes of the brain, since they lie very close to the undersurface of the cranial capsule. Dietary photosensitization syndrome may be an alternative explanation for sunburn in fish (Roberts, 1978b).

Poor nutritional status, such as vitamin and amino acid deficiencies, can result in a wide variety of lesions, which could confuse identification of toxicological pathologies. For instance, proliferative gill lesions are often observed after exposure of fish to water-soluble toxicants. However, a deficiency in pantothenic acid, a necessary constituent of coenzyme A responsible for normal fat and carbohydrate metabolism, causes a similar gill pathology. Lesions of this "nutritional gill disease" consist of lamellar epithelial hyperplasia with eventual fusion of secondary lamellae near the tips of gill filaments (Cowey and Roberts, 1978). Since lesions may result from poor nutrition, fish used in toxicological studies must be fed a nutritionally adequate diet free of any contaminant that might bias the results. If a toxicant is administered orally with the food, its effect on the palatability of the diet is important. Refusal to feed if the diet is off-flavored results in malnutrition and no exposure to the toxicant.

Congenital anomalies, presumed to be gene-mediated disorders, can occur in fish offspring. These conditions include vertebral deformities, anomalous fin development, shortened operculae, foreshortened or overextended jaws, polycystic conditions in viscera of trout, hypoplastic swim bladder in certain cyprinodonts and Siamese twins (Roberts, 1978c). Genetically induced neoplasia was observed when two closely related species of aquarium fish were artificially hybridized: platy (*Xiphophorus maculatus*) and swordtail (*X. helleri*) crosses developed malignant melanomas (Gordon, 1959; Sobel et al., 1975).

Lesions in fish resulting directly from primary metabolic disturbances have been described only for sexually mature individuals that were ready to spawn. Vascular lesions, consisting of intimal hypertrophy, cartilaginous metaplasia, and fibrosis, occur in Pacific (*Oncorhynchus* sp.) and Atlantic (*Salmo salar*) salmon and steelhead trout concomitant with drastic changes in sexual hormone levels during spawning migration (Robertson et al., 1961; Van Citters and Watson, 1968; Maneche et al., 1972). Interestingly, these lesions regress in the steelhead that survive the spawning ordeal. In Pacific salmon increases in serum levels of 17-hydroxycorticosteroids cause an increase in cell numbers and intracellular products in the pituitary and adrenal glands. These changes are indicative of increased organ function; however, at full maturity cells of the pituitary undergo de-

generative changes and the adrenal is hyperplastic (Robertson and Wexler, 1962a, 1962b). Hyperglycemia concurrent with hyperplasia of the endocrine pancreas (islets of Langerhans) may also occur (Love, 1970), as well as degeneration and atrophy of glomerular elements (Hay et al., 1976). Metabolic disturbances secondary to other primary causes are responsible for additional disease syndromes: sekoke disease of carp (*Cyprinus carpio*), initiated by silkworm pupae in the diet, is characterized by degranulation of B cells in the islets of the pancreas, causing diabetes mellitus accompanied by a retinopathy, thickening of the gill basement membranes, and telangiectasia of lamellar sinusoids (Yokote, 1974); goiter of the thyroid gland resulting from iodine deficiency (Cowey and Roberts, 1978).

Fish are immunologically competent, with cellular and humoral responses similar to those in higher vertebrates. Immediate type hypersensitivity reactions such as anaphylactic shock have been reported in various fish species (Dreyer and King, 1948), including goldfish (*Carassius auratus*) and channel catfish (*Ictalurus punctatus*) (Goven et al., 1980). It is likely that immune complex mediated or immunodeficiency diseases (and lesions) occur in fish; however, none have been reported as of this writing.

Infectious diseases are another major source of lesions, and close examination of tissues by use of histochemical techniques and microbiological procedures may be needed to eliminate bacteriological, viral, fungal, protozoal, or other parasitic etiologies. Because many of these pathogens are opportunistic, occurring as secondary invaders, the primary cause of a lesion may not be obvious despite the overwhelming presence of a particular infectious agent. Thus, caution must be exercised in interpreting lesions of this nature. An extremely helpful reference on necropsy and diagnostic procedures for infectious diseases of fish is provided by McDaniel (1979). Voluminous descriptive literature exists on infectious diseases of fish and resultant pathologies; the investigator is referred to Roberts (1978a) or Ribelin and Migaki (1975) for further information. Overstreet (1978) is also a useful source for disease studies of marine fish and invertebrates.

It becomes apparent from the discussion above that, although various factors other than toxicants can be primary initiators of disease and tissue pathology, a histopathologic etiology may involve two or more of these factors acting in a synergistic manner; for instance, genetic inbreeding can produce immune-deficient offspring that are more susceptible to infectious disease. This type of relationship emphasizes the complexities that face the aquatic pathologist in interpreting lesions and diagnosing disease.

GENERAL TYPES OF TISSUE CHANGES

General Inflammatory Process

Regardless of cause, there are certain fundamental changes that can occur in tissues, and these changes form the basis of descriptive pathology. Many of these changes are part of or result from the inflammatory response in the living animal. Inflammation is a dynamic series of events that occur only in viable tissues in response to an irritant. The biological function of the inflammatory response is to destroy or "wall off" irritating substances so that damaged tissue may heal. Many entities can act as irritants, causing tissue damage and necrosis (necrotic tissue itself is an irritant) eliciting an inflammatory response; examples are infectious agents (bacteria, viruses, protozoans, fungi, metazoans) and their toxic metabolites, mechanical injuries, chemical toxicants, inanimate foreign bodies, antibody-antigen complexes, sequestered antigens, infarcts, and loss of nervous innervation. The events that comprise the inflammatory response are essentially the same in all vertebrates and have been described in great detail (Zweifach et al., 1965; Smith et al., 1972). Classic early inflammation involves vascular and exudative stages accompanied, in higher animals, by the four cardinal signs of redness, swelling, heat, and pain. In lower poikilothermic vertebrates heat is not

likely to be one of the cardinal signs; however, the rate of the inflammatory process fluctuates directly with ambient temperature.

Inflammation begins following tissue damage, which initiates release of chemical mediators from resident mast cells and basophilic leukocytes. These mediators (histamine, serotonin, kinins, and so on) are vasoactive amines that affect vessels in the immediate area of injury by causing vasodilation and increased vascular permeability (enlargement of fenestrae in capillary walls). The vascular dilation allows an initial increase in blood flow (hyperemia causing redness and heat), while the more permeable vessel walls permit passage of serum proteins, erythrocytes, and migrating leukocytes (neutrophils, macrophages, lymphocytes) into nearby injured tissues (causing swelling). Fibrinogen, an important terminal component in the cascading clotting mechanism, is among the serum proteins that escape into the wound; it polymerizes (clots) to an insoluble protein, fibrin, which provides a fibrillar network for enhanced amoeboid migration of immunologically activated leukocytes. Continual loss of fluid from the blood and the adhesiveness of leukocytes finally cause a localized increase in blood viscosity with retardation of flow and eventual stasis. This passive congestion further enhances escape of humoral and cellular elements into the injured tissues. This exudative stage of inflammation is extremely important for tissue recovery because it is here that serum antibody and activated leukocytes of the immune system are liberated to attack the initial irritant (and its metabolites), necrotic host cells, and other deleterious products released by injured cells. These noxious substances and the exudative swelling irritate nerve endings, causing pain, the last of the cardinal signs.

Types of Inflammation

Inflammation is classified into various types according to the identity of the predominating elements in the exudate. Six types of inflammation have been recognized (see Terminology section for complete description of each): (1) serous inflammation, (2) fibrinous inflammation, (3 purulent inflammation, (4) hemorrhagic inflamma tion, (5) catarrhal or mucous inflammation, an (6) lymphocytic inflammation. Each type may b further classified according to duration an whether proliferation of new fibrous connectiv tissue has occurred. An acute inflammation is on that has a sudden clinical onset and progresse rapidly to recovery with no associated prolifera tion of connective tissue. A chronic inflammatio is one that begins imperceptibly and continues fo an indefinite period with resultant production o mature fibrous connective tissue. A subacut classification is intermediate, characterized b proliferation of new connective tissue that is no yet mature.

Results of Inflammation

The sequellae or outcome of inflammation in th host can be resolution, death, or continuation t chronicity. Resolution is characterized by com plete restoration of tissues to their normal appear ance and function by local proliferation of th necessary specific cell types (regeneration). I many acute inflammations, the host response i adequate in quickly removing the irritant, which i unable to cause extensive tissue injury. The degre of acute response is usually dependent on hov well the host is able to recognize the irritant a nonself. This is also true for poikilotherms excep as mentioned before, the efficiency of foreig body recognition is dependent on optimur ambient temperatures, which vary among specie

In some cases acute inflammation is unable t destroy an injurious agent, and there is sever damage to vital tissues, followed by death. I extremely acute infectious diseases or toxican exposures, death can occur so rapidly that tissu injuries appear insignificant and inflammator changes have not yet had time to occur. Micr scopic interpretation of such tissues may provid little information for diagnostic purposes.

Another possible outcome is that inflammatio is unsuccessful in completely eliminating the initi ting agent, which spreads and causes continuo

and sometimes extensive tissue damage—a situation that allows little or no regeneration of parent tissue cells. This inadequate response often leads to a chronic inflammation with proliferation of new tissue, usually by fibrosis, as an attempt to wall off the irritant and replace lost cells. Since fibrous connective tissue can function only in a supportive manner, the specialized functions of replaced cells are lost. This process can continue indefinitely and may result in eventual death of the host due to replacement of vital tissues by the lesion.

The formation of fibrous granulomata is an example of a chronic proliferative inflammation that is quite common in fish and is usually caused by certain bacteria, fungi, or noninfectious foreign bodies. Early in development, these lesions are characterized by acute inflammation. This is followed by chronic multifocal proliferations, each of which appears as a central zone of necrotic cellular debris, often containing the causative agent, surrounded by a layer of epithelioid cells (macrophages resembling epithelial cells) interspersed with other leukocytes with encapsulation of the entire focus by new fibrous connective tissue. Another type of granuloma is formed principally by reticuloendothelial cells, which appear as small foci of proliferating macrophages or histiocytes, usually circumscribed by a collar of lymphocytes.

The inflammatory response can be excessive rather than adequate or inadequate. The inflammation may be able to eliminate or compromise the initiating irritant, but the severity or anatomic location of the response may threaten to kill the host before this can be accomplished. For example, exposure of fish to various infectious agents or toxic materials results in an exuberant proliferative inflammation of the gills. This is characterized by some infiltration of leukocytes and proliferation of connective tissue elements, but the outstanding feature is epithelial hyperplasia that causes fusion of secondary lamellae and clubbing of gill filaments. This loss of respiratory surface may result in death by anoxia before resolution of the initial insult.

Degenerative Changes in Cells

Cellular degeneration and death (necrosis) are not only fundamental to the inflammatory process but form the basis of many other descriptions in pathology. Most degenerative changes in cells are initiated by inability to maintain proper ionic balance. Cell injury causes an intracellular reduction in oxidative phosphorylation with a resultant drop in the level of adenosine triphosphate (ATP) and a concomitant shift toward acidosis (pH 6.0) due to glycolysis. The cation pump of the cell, which uses ATP as an energy source, breaks down, allowing an influx of sodium, chloride, calcium, and water. This causes cellular swelling and damages cell membranes, which leak intracellular ions (potassium), enzymes, and other proteins. Most degenerative changes in cells are reversible to a certain degree before cell death becomes inevitable. A condition known as acute cellular swelling, or cloudy swelling, is the earliest form of cellular degeneration and is often followed by a more advanced stage known as hydropic degeneration (vacuolar degeneration). Other types of degenerative changes in cells (see Terminology section) include fatty change, hyalin degeneration, fibrinoid degeneration, mucoid degeneration (i.e., serous atrophy of fat or myxomatous degeneration), and ballooning degeneration.

Necrosis

There is a point at which degenerative changes within a cell become irreversible, terminating in cell death or necrosis while the rest of the body still lives. This is especially true when intracellular membranes become damaged, allowing hydrolytic enzymes to leak out from ruptured lysosomes to further destroy cell integrity. Postmortem autolysis (necrosis after death of the body) is similar but is usually more generalized and lacks the vascular and cellular responses (inflammation) that occur around necrotic tissues during life. Necrotic changes in the nucleus determine whether cell death has occurred. These changes include pyknosis, karyorrhexis, karyolysis, or absence of

the nucleus (except for the normally anucleate erythrocytes and thrombocytes of mammals). Less reliable features of necrosis include cytoplasmic changes such as increased acidophilia and cytoplasmolysis (dissolution of cytoplasm), which can also occur in living cells. Additional necrotic changes are characterized by loss of cell outlines and loss of differential staining between cytoplasm and nucleus or among different cell types. If a cell is detached or absent from its normal surface or location, it is assumed to have died. For example, desquamated epithelial cells in the gut lumen may stain normally, but by virtue of physical separation from parent tissues these liberated cells cannot survive and must be considered dead.

The specific causes (listed in the discussion of inflammation) of cellular injury and the kind of tissue in which it occurs determine the microscopic appearance of the necrotic process, thus forming a basis for classification. Five general types of necrosis (described in Terminology) are recognized: (1) coagulative necrosis; (2) caseous necrosis; (3) liquefactive necrosis; (4) fat necrosis; and (5) Zenker's necrosis.

Tumors/Neoplasia

Another tissue change that is fundamental in pathology is neoplasia, or tumor formation. Neoplasia is the pathological transformation of a normal host cell resulting in uncontrollable proliferation into new cells which resemble the original host tissue but lack normal histological architecture or normal cellular functions. The uncontrollable growth and the lack of contact inhibition make cancer an extremely destructive process. Proliferation of neoplastic cells can occur in two ways. A tumor can grow by expansion, causing compression atrophy of normal tissues outward from the enlarging focus of neoplastic cells. This is characteristic of a benign tumor, which generally is less destructive unless it occurs in a location where it compromises life functions (e.g., the brain). The other growth process is infiltration of neoplastic cells into and around normal tissue structures. This process, which is characteristic of malignant tumors, is the most damaging one. By competitive exclusion of nutrients and oxygen, infiltrating malignant cells replace normal tissues, which are crowded out and disappear through necrosis. Eventually, malignant cells may invade a lymphatic channel or blood vessels, where clumps of the cells are likely to break off as emboli in the lymph or blood flow. These cells settle out in other organs and tissues and may proliferate into secondary malignant tumors. This proliferative dissemination of neoplastic cells from the primary site of growth is known as metastasis and is typical of most malignant tumors. Whether a tumor is benign or malignant is determined by histological examination. Cells of benign tumors are usually well differentiated and may be almost normal in architectural arrangement, closely resembling the tissues of origin. They usually show no tendency to infiltrate adjacent normal tissues, never metastasize, and are usually circumscribed or encapsulated with fibrous connective tissue. Invasive growth is rarely seen in certain types of tumors that are considered benign because they do not metastasize (e.g., lymphangioma of humans, equine sarcoid, basal cell carcinoma of humans and dogs). Besides erosive, infiltrative growth and metastasis, malignant tumors have other characteristics, which are used for classification if invasiveness is not apparent: (1) cells may be very anaplastic in that they appear undifferentiated and immature, having little or no recognizable arrangement to permit identification of the parent tissue; (2) in glandular tumors cells tend to "pile up," forming papillary projections in the lumina of acini; (3) tumors show increased cellularity, indicated by large numbers of closely spaced nuclei per unit area; (4) appearance of hyperchromatic, plump nuclei; and (5) a high mitotic index and/or the presence of abnormal mitotic divisions.

The prognosis or probable outcome of a neoplastic disease depends not only on whether the tumor is benign or malignant but also on its clinical behavior. Some malignancies do not grow rapidly, and metastasis occurs late, after tumor

development is well advanced (e.g., hepatocarcinoma in rainbow trout). In these cases survival of the host is prolonged.

In vertebrates, nearly all living, dividing populations of cells comprising different types of tissues are capable of falling victim to the neoplastic process. This section is not intended to give detailed descriptions of tumor types, but rather to acquaint the investigator with the concept of neoplasia and the terminology used in its classification. For further information on neoplasia, the reader should consult Smith et al. (1972), Robbins (1974), and Moulton (1978). Tumors are classified according to the host cell(s) of origin belonging to one of the three basic categories of tissue: epithelium, supporting tissue (connective tissue, muscle, bone, cartilage, blood, and lymphoid tissue), and nervous tissue. Exceptions are mixed tumors and teratomas, which may consist of cell types from two or all three of the categories, respectively. Most epithelial tumors fall in three general classes: papillomas, adenomas, and carcinomas. Several other specialized tumor types exist but will not be mentioned here. A papilloma is a benign outgrowth of epithelium and is usually a complex arrangement of fingerlike projections with a fibrous connective tissue core. An adenoma is a benign epithelial tumor that forms acinar-like glandular structures, although tumors of nonacinar glands are also included. If the glandular epithelium of the adenoma, say of the pancreas, exhibits malignant characteristics, the tumor is reclassified as an adenocarcinoma of the pancreas. Therefore, the term "carcinoma" is used to denote malignancy in an epithelial tumor; another example is a squamous cell carcinoma.

Benign tumors of supporting tissues are classified similarly, with the suffix "oma" used after the tissue of origin: fibroma and chondroma refer to benign tumors of fibrous connective tissue and cartilage, respectively. The term "sarcoma" indicates a malignant tumor of supporting tissue origin and is used as a suffix, as in fibrosarcoma or chondrosarcoma.

Tumors of nervous tissue origin can occur in the central nervous system (brain or spinal cord) and the peripheral nervous system (all other nervous innervation of tissues and organs). Most notable are tumors of the brain and spinal cord, most of which are of neuroglial origin. These "gliomas" are of several types, conforming to the classes of neuroglial cells. Depending on the tumor type, gliomas have different rates of growth and locally invasive properties characterized by the terms benign and malignant. However, even a benign tumor of the brain or spinal cord can be destructive and life-threatening because of the fragile nature of nervous tissue and the specific location of the growth. Interestingly, malignant gliomas are not known to metastasize outside the cranial cavity or spinal canal (Smith et al., 1972; Robbins, 1974).

The suffix "blastoma" is often encountered in tumor terminology, as in neuroblastoma (tumor arising from nervous tissue during embryonal development), nephroblastoma (tumor of renal blastema from which kidney is formed), and ameloblastoma (tumor of the enamel organ, the embryologic predecessor of the tooth). As one might surmise from these definitions, blastoma refers to primary tumor formation in immature tissues encountered during embryonal development rather than in mature adult cells.

Causes of Neoplasia

Depending on the life span of the host species, tumor induction may have a latent period of months, years, or even decades. Unfortunately, the mechanisms and circumstances involved in transformation of a normal cell to one that is neoplastic are not well understood. Nonetheless, some factors have been identified as causing cancer. Overexposure to ultraviolet light from the sun or artificial sources is known to cause skin carcinomas in humans and certain bovine breeds. More destructive forms of cancer-causing irradiation are present in x-rays and in radioactive isotopes such as radium, thorium, and cobalt 60. Chemical carcinogens such as derivatives of 1:2-benzanthracene are perhaps the best known tumor-causing agents

since many have been discovered through testing in a wide variety of laboratory animals, including fish. Hormones have also been implicated in cancer formation. For example, large doses of estrogenic hormones administered to certain laboratory animals have resulted in hyperplasia and neoplasia in organs normally under hormonal control. Infectious agents also play a role in tumor induction. Among metazoan parasites, the nematode *Spirocerca lupi* may cause malignant tumors in the esophagus of the dog. Oncogenic properties of some viruses have also been well documented with descriptions of papillomas, adenocarcinomas, fibromas, sarcomas, and lymphomas induced by viruses in avian and mammalian species.

The effects on an individual animal of each of the cancer-causing entities listed above are influenced by a unifying variable, heredity. That is, the relative susceptibility of an animal, or of an individual tissue in the animal, is genetically determined. For example, certain strains of mice may be highly resistant to induction of any tumor type, while other strains are susceptible to one or several kinds of tumors.

VARIABLES IN THE DEVELOPMENT OF TOXIC LESIONS

Many studies have shown that the lethality of a particular toxic compound varies depending on the age of the animal as well as the dose (or concentration) and time of exposure. Lesion development may be enhanced by larger doses, longer exposure periods, or use of juvenile experimental animals, which tend to be more susceptible to toxicant-induced insult than adults. The solubility of a toxic compound and its route of exposure may also influence the kinds of lesions observed. For example, compounds that are water-soluble or have water-soluble toxic components may be easily absorbed into the blood of fish or shellfish through direct contact with the respiratory epithelium of the gills or epidermal and sensory epithelium of the skin and buccal cavity. Therefore, it is not uncommon to find initial lesions involving the gills and epidermis, including the sensory epithelium of the olfactory and lateral line organs. However, water-soluble toxicants may not cause external epithelial lesions, but may be absorbed and produce systemic pathology elsewhere in the organism. Some of these compounds may become toxic only when altered by cellular metabolism. In saltwater fish, additional contact absorption of dissolved compounds and hence lesions can occur in the alimentary tract because these fish drink seawater to maintain osmotic equilibrium.

Chemical compounds that are not water-soluble or are only slightly soluble require other routes for cellular exposure. Many such compounds are soluble in lipids and are concentrated to toxic levels in the adipose tissues of prey animals. When such prey are eaten, fat digestion in the predator may release a lethal or, more likely, a sublethal dose of the toxicant that can produce chronic internal lesions during long periods of repeated low-level exposures. Experimentally, some water-insoluble toxic compounds can be dissolved in a carrier solvent and administered orally (directly into the mouth or in the diet) or parenterally (by injection). In either case, external lesions are uncommon. When interpreting lesions, the investigator should be aware that parenteral injections may also produce localized pathological changes due to trauma or a host-foreign body response (inflammation, fibrosis, and organ adhesions) to the carrier solvent. Examination of control animals given "sham" inoculations (injections without toxicant) prevents confusion of these artifacts with lesions resulting from the toxicant.

METHODOLOGY

Introduction

There are no standardized techniques for examining tissues in aquatic organisms. However, standard medical and veterinary techniques may be modified and used to diagnose tissue changes in fish and other aquatic organisms. Before beginning an

experiment it is necessary to plan and coordinate the efforts involved, because histopathology is often only one of several types of information obtained from the experiment. The variables that should be considered in the experimental design include the most desirable animal species, numbers of animals, age of animals, diet, means of housing the animals, route of exposure, doses (or concentrations), length of exposure, effluent disposal, sampling schedule, tissues to be examined, tissue fixation (which is highly variable and depends on the techniques to be used), tissue processing, staining, and interpretation. The following are the general procedures followed in investigating toxicant-induced tissue changes or carcinogenesis in fish at the Food Toxicology and Nutrition Laboratory (FTNL) at Oregon State University.

Fish Methods

Species, Diet, Holding Conditions

The principal research animal used at FTNL is the Shasta strain of the rainbow trout, *Salmo gairdneri*. It has proved to be sensitive to many toxic compounds, is easy to rear in our laboratory, and, because of its large size, provides sufficient tissue (particularly liver) for *in vitro* metabolism studies. However, trout require large holding facilities and exacting criteria for water quality, and not all laboratories can conveniently rear them. Several other fish species are also used for toxicological research, including the channel catfish *Ictalurus punctatus*; goldfish *Carassius auratus*; carp, *Cyprinus carpio*; and bluegill, *Lepomis macrochirus*. More recently, several species of small aquarium fish have become popular in research. These include the Japanese medaka, *Oryzias latipes*; guppy, *Lebistes reticulatus*; zebrafish, *Brachydanio rerio*; fathead minnow, *Pimephales promelas*; mummichog, *Fundulus heteroclitus*; and sheepshead minnow, *Cyprinodon variegatus* (the latter two are estuarine species). These small species have the obvious advantages of low maintenance costs and smaller facility requirements. However, *in vitro* studies are limited by the smaller size of the species.

Rainbow trout at FTNL are fed a semipurified casein test diet (Sinnhuber et al., 1977), which supports adequate growth and permits reproducibility of experiments. Many commercial diets vary considerably from year to year, depending on availability and cost of the ingredients, which makes them undesirable for experimental work. The semipurified diet also facilitates incorporation of toxic materials since it is prepared as needed in the laboratory rather than premixed and pelleted like commercial rations.

The trout are reared in circular fiber glass tanks that receive an adequate flow of oxygenated, constant-temperature (12°C) well water. Groups of 100 fingerling trout may be held for up to 1 yr in a 4-ft-diameter (200-gal) tank, but for only 9 mo in a 3-ft (100-gal) tank, without overcrowding and stunting of growth. The standard procedure is to start a group of 100 fingerlings in a 3-ft tank, reduce the number through sampling to 60 at 9 mo, and terminate the experiment at 12 mo. If it is desirable to extend the experiment to 18 or 24 mo, a sample of 20 fish would be taken at 9 mo and the remaining 80 fish transferred to a 4-ft tank. Another 20-fish sample would be taken at 12 mo and the remaining 60 fish held in the 4-ft tank until 18 mo or more. Duplicate groups of fish for each experimental regime are routinely used to provide increased animal numbers and insurance against complete loss of data if water failure causes mortalities in one tank.

Exposure and Concentrations

Several routes of exposure to toxic chemicals are available when using rainbow trout or other fish species. These include diet, flow-through water, static water, and injection.

Many of the exposures to toxic chemicals at FTNL have been by the dietary route. This has proved convenient and effective since many of the compounds (aflatoxins, chlorinated hydrocarbons, and polyaromatic hydrocarbons) are highly lipid-soluble and easily added to the fish oil fraction of the diet. Depending on the experiment, a continuous low-concentration feeding exposure or

sometimes a short-term, high-concentration, pulse-feeding exposure may be used. The main disadvantage of dietary exposure is that individual fish have unequal feeding rates and hence receive uneven concentrations of toxicant. A pecking order or dominance structure is established in each tank of fish, so that some fish eat more and some much less at each feeding.

Exposure of fish to low levels of toxicant dissolved in the water best represents their exposure in the environment. Thus, to determine the effects of environmental pollutants on fish, this route of exposure should be used. If fish are being used as a model system to test the toxicity or carcinogenicity of a compound in general, other routes of exposure, which require less elaborate facilities, are more desirable. The main disadvantages of flow-through exposure are that elaborate diluting and monitoring systems are required to deliver a constant concentration of toxicant, and a satisfactory waste treatment system is needed to dispose of the toxicant.

Static water exposures of fish to toxicants have been used extensively for short-term LC50 determinations. This method has advantages and disadvantages when compared to the flow-through method. Facility requirements are minimal and treatment and disposal of waste water are much easier, but length of exposure is limited. An adaptation of this method that is gaining popularity as a carcinogenicity test system is static exposure of fish embryos (eggs) to solutions of carcinogens (Hendricks et al., 1980d). It has proved effective with several mycotoxins and nitrosamines and the advantages over feeding exposures are that (1) it requires only a single handling of the carcinogen; (2) there is passive, uniform exposure of each egg to the carcinogen; (3) a small amount of carcinogen will expose large numbers of eggs; (4) detoxification and disposal of the carcinogen are convenient; and (5) in several cases, embryos have proved to be more sensitive to carcinogens than fingerlings (Wales et al., 1978). The main disadvantage is that it may be difficult or impossible to expose fish embryos to sufficiently high concentrations of highly water-insoluble compounds.

Few long-term toxicity or carcinogenicity experiments use injection as the route of exposure; the main objection is to the continual stress and trauma placed on the fish by repeated handling. Injection can, however, be used effectively if care is taken and fish are of sufficient size to withstand the injection volume.

Regardless of the route of exposure, it is preferable to conduct short-term trials to determine the maximum tolerated concentration (MTC) of a compound before concentrations are selected for a long-term trial. Knowledge of the MTC and the nature of the compound will facilitate the selection of three to five concentrations, with the MTC as the high concentration, for a concentration-response study. If there are budget or space restrictions, a single concentration corresponding to the MTC may be used. In either case a negative control group is reared to ensure that the observed effect is due to the toxicant.

Sampling

The next variables to establish are the number of fish to sample and when to sample them to give the greatest amount of information. Depending on the objectives of the study, an experiment usually begins with duplicate groups of 100 trout for each concentration of a compound and controls. If the experiment is to run for 12 mo, random samples are taken of 20 fish at 6 mo, 20 fish at 9 mo, and 60 fish at 12 mo. If the experiment is to last 18 mo, the 20-fish samples will be taken at 6 or 9 mo and 12 mo and 60 fish at 18 mo.

Data Collected

The following data and tissue samples are routinely taken at each sampling date described above: individual body weight (growth), feed efficiency (diet consumption, recorded throughout the experiment), individual liver weight, ratio of liver weight to body weight, tissues for bioaccumulation analysis (three or more fish per group, killed and frozen whole), gross observations

of external or internal lesions, and target tissues for fixation and later histopathologic examination.

The liver is often the target organ for many of the toxicants tested. However, if a toxicant is known to affect other organs, additional tissues may be fixed for observation; examples are mesonephric kidney, head kidney, alimentary tract, pancreas, spleen, thyroid, thymus, heart, gonads, swim bladder, gill, and brain. If a new compound is being tested for the first time, all tissues are fixed.

Tissue Fixation, Processing, and Staining

Light microscopy Toxicant-induced injuries in fish tissues are often difficult to discern grossly (except for large tumors); light microscopy is necessary to observe lesions at the cellular level. The following discussion concerns effective procedures for necropsy of fish specimens, tissue fixation, and histological preparations useful in light microscopy. Fish can be immobilized, a few at a time, by an overdose of tricaine methanesulfonate (MS 222) anesthetic or knocked unconscious by a sharp blow to the head. When all physical activity ceases, the fish are examined for external lesions, weighed individually, and one or more of the gill arches is cut to permit bleeding. The beating heart quickly pumps most of the blood from the body. This facilitates easy observation and subsequent removal of tissues. The body cavity is opened by a midlateral incision from the vent to the operculum and the liver is quickly removed by severing the anterior hepatic veins and the posterior portal vein and bile duct. Care must be taken to prevent spillage of bile from the gallbladder since bile causes necrosis of most tissues, especially liver. The liver is weighed and observed under a dissecting microscope for gross abnormalities before it is placed in a suitable fixative, usually Bouin's solution. Other organs are then removed, gross abnormalities noted, and portions fixed as above.

Before satisfactory histological sections can be produced from a tissue, attention must be given to its preparation. Proper fixation is fundamental. The rapid autolysis of tissues after death means they must be handled rapidly to prevent degenerative changes in the specimen. This process is halted by fixation, which preserves the morphology of tissues in a condition as near as possible to that existing during life. Many simple and compound fixatives are used for light microscopy. The most common example of the former is neutral buffered formalin. Compound formaldehyde-containing fixatives include Bouin's solution, Helly's fixative, Orth's fixative, and Susa's fixative. Common fixatives that do not contain formaldehyde are Carnoy's and Zenker's fixatives. Liberal amounts of fixative, relative to the mass of tissue (at least 10:1), should be used to ensure rapid and complete fixation. Tissue specimens should be kept small ($\leq$1 cm thick) to promote rapid penetration.

Depending on the preservative, tissue fixation requires 18–24 h; longer periods may be needed to make optimal preparations. After fixation is complete, the tissues are dissected and trimmed into acceptable sizes for processing and sectioning. They are put in small carriers and washed in running water before being placed in an automatic tissue processor for overnight dehydration, clearing, and paraffin infiltration. This machine, which operates on a timer, moves the tissues through a series of increasing concentrations of ethyl alcohol (70–100%), two or three changes of xylene (a clearing agent), and two changes of melted paraffin. In the morning, the tissues are embedded in molds containing clean melted paraffin and these are allowed to harden at room temperature. The hardened blocks are trimmed, mounted on tissue chucks, and sectioned on a microtome into ribbons at a thickness of 4–6 microns. Selected sections are floated and stretched on a hot-water bath, mounted on clean glass slides, and placed on a warming tray to dry and adhere.

At this point the tissues are ready for staining. For most routine histopathology, slides are stained with the nuclear cytoplasmic stain hematoxylin and eosin (H&E), which renders the nuclei blue and the cytoplasm pink. H&E is usually sufficient, but sometimes other stains are required to demon-

strate or identify certain tissue or cellular components, abnormal structures, or inclusions. For further discussion of specific techniques the reader is referred to several excellent references (Luna, 1968; Humason, 1962; Putt, 1972; Lillie, 1965; Pearse, 1961; Thompson, 1966). Journals such as *Stain Technology* and the *Journal of Histochemistry and Cytochemistry* are good sources of specific techniques.

Histochemistry Histochemical techniques may be required to investigate the presence of certain chemicals (fat, carbohydrates, protein enzymes) in tissues. These techniques require different fixation procedures from routine light microscopy. For instance, to demonstrate glycogen with periodic acid–Schiff (PAS) stain, it is necessary to fix the tissue in an alcohol-based fixative (alcoholic formalin or Bouin's) to prevent dissolution of the water-soluble glycogen. For lipid stains such as Sudan IV and Sudan Black B, aqueous fixatives are used, preferably 10% formalin. If enzyme histochemical procedures are indicated, fresh frozen or formalin-fixed tissues are used for incubation procedures.

Transmission Electron Microscopy Histological examination of tissue sections from fish after acute or low-level chronic exposure to chemicals may not reveal any obvious lesions. However, examination of the ultrastructural features of the same tissue specimens may reveal significant pathological changes of cells or cellular organelles which could explain physiological or morphological irregularities not manifest at the light microscopic level.

For transmission electron microscopy (TEM), fixation in 2–5% glutaraldehyde in 0.1 *M* phosphate or cacodylate buffer (pH 7.4) is done overnight at 4°C, after which tissues are removed and postfixed to increase electron density in buffered 1–2% osmium tetroxide for 1–3 h at 4°C. (cellular fixation is qualitatively enhanced and integrity of the dilute fixative is best preserved at low temperatures) (Pease, 1964). Postfixed tissues are rinsed in buffer and dehydrated through a series of ethanol baths (70–100%), with two or three final changes of 100% ethanol for 5–10 min each to ensure complete dehydration. In this way there should be no problems later with infiltration and proper hardening of the resin blocks. If storage is desired, postfixed specimens rinsed in buffer may be kept indefinitely in 70% ethanol for later processing. Dehydrated tissues are placed in a mixture of 50% Spurr's low-viscosity resin (Spurr, 1969) and 50% absolute ethanol and allowed to infiltrate on a rotator overnight at room temperature. Complete resin infiltration is accomplished the next day by rotating the tissues in undiluted Spurr's resin for 4–6 h. Embedment is completed by adding tissues and fresh resin to BEEM capsules or other suitable plastic molds and placing them in an oven at 70°C. Spurr's resin cures in about 8 h, after which the molds may be peeled away to allow trimming and sizing of the block faces for sectioning. Trimmed blocks are placed in an ultramicrotome and thick sections (0.5–1.0 μm) are first cut for examination by light microscopy to determine if the desired field is present in the block for thin sectioning. Thick sections are floated on a water reservoir behind the cutting surface of the knife (glass or diamond) and placed on a clean glass slide with a small wire loop. The slide is heated over an alcohol flame for drying and adhesion of the sections to the glass surface. These sections are then deplasticized with a saturated solution of NaOH in absolute ethanol for 2–5 min (depending on thickness), rinsed in absolute ethanol and distilled water, and stained with toluidine blue (2% solution buffered with 2% sodium tetraborate) while heated on a slide warmer at 60°C. Staining time also varies with the thickness of the section and is usually 2–5 s. Stained slides are rinsed in distilled water and coverslipped with Permount. Cell nuclei stain purple-pink and cytoplasm is blue.

Thin sections (250–500 Å) are cut similarly, except that they are mounted on cleaned tiny copper meshed grids. This is done by gripping the grid with forceps and bringing it directly up under the floating section for proper seating. The grid and section are allowed to air-dry before staining

with uranyl acetate (30 min) and counterstaining with lead citrate (2–5 min). Grids are then ready for viewing of cellular structure in the electron microscope.

The description above is a summary of a basic procedure applicable to many situations in TEM. However, there are several variations involving fixatives, buffers, stains and staining sequences, technique, embedding resins, and so on which may be more suitable for a specific need. More detailed descriptions of the procedures can be found in textbooks on TEM (Pease, 1964; Hayat, 1972, 1977) and in the literature (Hawkes, 1977, 1980).

Scanning Electron Microscopy Scanning electron microscopy (SEM) is useful for observing three-dimensional ultrastructural changes on the surfaces of tissues, cells, or other structures. Small tissue samples are preserved in a fixative, postfixed as above, and dehydrated through a series of acetone baths (25, 50, 75, and 90%). Dehydration is complete after two or three immersions in pure acetone for 5–10 min each. Dehydrated samples are dried in a special manner to prevent structural distortion due to pressure exerted by the surface tension of evaporating fluids, which normally occurs in air-drying. However, air-drying may be satisfactory for hard tissues such as chiton, shell, and bone. Soft tissues are dried in a critical-point dryer which, under pressure, replaces the acetone bath surrounding the specimens with liquid CO_2 or some other pressurized gas. The CO_2 is vaporized by increasing the pressure of the reaction vessel to the critical point (800–850 lb/in^2 or 1870 K/6.25 cm^2) by immersion in a hot-water bath. The gas is released slowly and the dried specimens are carefully removed for the next step. The specimens are mounted on an aluminum stub with double-sided tape or silver conducting paint. The stubs are placed in a vacuum evaporator which coats the surface detail of each specimen with a thin layer of metal dust by vaporizing a fine gold:palladium (60:40) wire. Stubs holding the coated specimens are removed from the evaporator and are ready for insertion into the scanning electron microscope. The metal dust coats the finest surface detail and conducts electrons from a focused electron beam, which produces an image of the specimen on a monitor screen. Again, this is a simplified version of a procedure that may vary depending on the results desired and the equipment available; critical-point dryers differ in the gases used and different metals may be employed to coat specimens. For further details on this protocol and others, see Hayat (1972, 1977) and Anderson (1969).

Invertebrate Methods

In most cases, many of the techniques used for fish tissues are applicable to invertebrates; however, these organisms are considerably more diverse in tissue, body structure, and physiology (Morton, 1971) than fish, so the investigator should regard this statement as a rule of thumb. For discussion, bivalve mollusks (oysters, scallops, mussels, clams) will be used as an example. Here the investigator must extract tissues for examination from between two opposing, often formidable, shells (valves). This is easily done by inserting a knife and severing the adductor muscles (one in oysters and scallops and two in mussels and clams), which hold the valves tightly closed. Histological procedures used for adult invertebrate tissues are similar to those outlined earlier for fish tissues. Many of the conventional histological and histochemical stains can be used effectively on paraffin-embedded invertebrate tissues, with H&E providing good routine microscopic detail (Buchanan, 1970; Meyers, 1981). Histological or histochemical procedures requiring frozen sections also work well in mollusks (Liddell, 1967; Meyers, 1979). Although the same general fixatives can be used, special attention should be given to the osmolarity if the animal was taken from salt water. Most invertebrates are conformers rather than regulators, which means that their cells are bathed in physiological fluids having a salt concentration approximating the salinity of ambient seawater. As with any tissues, this osmolarity should be maintained during fixation; adjustments can be made by adding NaCl or by preparing the

fixative with salt water approximately equal in salinity to that from which the animals were taken. Difficult problems of fixation can result with larval mollusks that are too small (<2-3 mm) for tissues to be extracted with a scalpel. These animals must be fixed while in their valves, and all too often these are closed immediately or soon after addition of the fixative to the seawater. This results in poor penetration of the fixative and unsuitable preservation of cellular structure. Premature valve closure can be overcome by narcotizing the larvae with an anesthetic before addition of the fixative.

Because of the calcareous nature of the valves, decalcification of larvae is required before embedment. This can be accomplished in 10% formic acid adjusted to pH 4.5 with sodium citrate. Decalcified larvae should be embedded in Spurr's resin or other plastic media for routine light microscopy, since paraffin does not infiltrate the small larval samples as well. Although thick sections of plastic- or resin-embedded materials limit the use of various histological stains, easily stained frozen sections may be prepared in parallel from decalcified larvae embedded in cryosectioning medium (O.C.T. Compound, Lab Tek Products). An excellent treatise on fixation and decalcification problems in larvae as well as definitive descriptions of additional microtechniques and larval structures in invertebrate histology and TEM are presented by Elston (1980a, b).

Adult invertebrate tissues are prepared for TEM with procedures similar to those used for fish, except that osmolarity is again critical. A solution that works extremely well in providing proper ionic balance when mixed with glutaraldehyde is Millonig's phosphate buffer (pH 7.6). Larval samples that have been embedded in plastic resins for light microscopy are equally suitable for ultrasectioning and TEM, depending on the fixative and type of resin used. Additional information on procedures for TEM of adult mollusks is given in Perkins and Menzel (1967) and Perkins (1968, 1975). Procedures for SEM present no problems that are unique for invertebrates other than that discussed for proper fixation in larvae. Further information regarding protocol is provided by Elston (1980a,b). For general references on invertebrate histology, ultrastructure, physiology, and biochemistry, see Malek and Cheng (1974), Galtsoff (1964), Sparks (1972), and Johnson (1980).

By using light microscopy, TEM, and SEM, the pathologist can observe, classify, and photographically document the lesions produced by a toxicant. In this chapter we have reviewed gross, histological, and ultrastructural lesions that have been described in aquatic animals exposed to toxic materials. These examples illustrate the types of lesions that can be expected.

LITERATURE REVIEW

The aquatic medium is a very efficient solvent for many chemical compounds or components thereof. Consequently, aquatic organisms are extremely vulnerable to toxic effects resulting from absorption or oral intake of these contaminants from the immediate environment. Various chemical compounds have been investigated to determine their potential toxicity to certain aquatic organisms, especially fish and to a lesser degree shellfish. However, many of these studies have been concerned with measuring lethality rather than the pathological effects of contaminants on the tissues. Table 1 lists several chemicals that have been examined for their potential to produce pathological changes in aquatic species. These aquatic species, along with specific toxicants and literature sources, are recorded in Table 2. Most of these studies employed an ambient water route of exposure, although some utilized per os (po) (through the mouth by incorporation into diet) or parenteral administration as well.

Nonspecific versus Diagnostic Lesions in Aquatic Toxicology

Unfortunately, toxicological studies of aquatic organisms have not revealed many tissue pathologies useful in diagnosing exposures to specific

Table 1 Organic and Inorganic Environmental Contaminants and Chemotherapeutic Agents Tested for Ability to Cause Pathological Changes in Tissues of Finfish and Shellfish

Organochlorines	Petroleum compounds	Organo-phosphates	Carbamates	Miscellaneous	Metals and nitrogenous compounds	Chemotherapeutic agents
Insecticides Chlordane DDD DDT Dieldrin Endosulfan Endrin Heptachlor Kepone Lindane Methoxychlor Mirex Toxaphene Herbicides Dowpon Dicamba Dichlobenil Diuron Dowicide G Esteron 2,4-D Kuron Kurosal Tordon 101 Tordon 22k Industrial Chlorinated wastewater Polychlorinated biphenyls (PCBs) Aroclor 1248 Aroclor 1254 Miscellaneous PCBs Carbon tetra-chloride	Crude oil Whole Saltwater-insoluble Saltwater-soluble Waste motor oil Oiled sediment Oil slick Miscellaneous hydrocarbons Miscellaneous hydrocarbons and PCBs Naphthalene Phenol	Insecticides Abate Diazinon Dimethoate Dursban Dylox Fenthion Malathion Methyl parathion	Insecticides Carbaryl (sevin) Propoxur	Herbicides Acrolein Amitrole-T Atrazine Dinoseb Diquat Hydrothol 191 Paraquat-CL Sodium trichloro-acetate ($NaTA_2$) Trifluralin Other Bis(tri-*n*-butyltin) oxide (molluscicide) Dimethyl sulfoxide (DMSO) Sodium lauryl sulfate (SLS-detergent) Triethylene phosphoramide (TEPA-insect chemosterilant) 3-Trifluoromethyl 4-nitrophenol (TFM-lampreycide) Acid-alkaline pH (sulfuric acid; sodium hydroxide)	Ammonia (NH_3) Cadmium-109 ($^{109}Cd^{+2}$) Cadmium chloride ($CdCl_2$) Copper chloride ($CuCl_2$) Copper nitrate ($CuNO_3$) Copper sulfate ($CuSO_4$) Landfill leachates Lead acetate trihydrate ($C_4H_6O_4Pb$) Lead nitrate ($PbNO_3$) Mercuric chloride ($HgCl_2$) Methyl mercury chloride (CH_3HgCl_2) Nitrite (NO_2) Silver chloride (AgCl) Sodium arsenate (Na_2HAsO_4) Sodium arsenite ($NaAsO_2$) Zinc chloride ($ZnCl_2$) Zinc sulfate ($ZnSO_4$)	Bradophen (disinfectant) $CuSO_4$ (fungicide, algicide) Dipterex Emtrysidina Formalin (fungicide, ectoparasiticide) Hexa-ex (disinfectant) Kanamycin Malachite Green (fungicide, ectoparasiticide) Methylene Blue (fungicide disinfectant) Neguvon, Masoten Ozone (biocide) Penicillin G Procain Dihydrostreptomycin sulfate Oxytetracycline Sulfamethazine Sulfonamides Thiabendazole Tobramycin Yomesan (anthelmintic)

Table 2 Aquatic Species Examined for Pathological Changes in Tissues following Exposures to Organic and Inorganic Environmental Contaminants and Chemotherapeutic Agents

Common name	Scientific name	Literature source
		Trout
Brook trout	*Salvelinus fontinalis*	Daye and Garside, 1976 (alkaline-acid pH); Sangalang and O'Halloran, 1972 ($CdCl_2$).
Brown trout	*Salmo trutta*	King, 1962 (DDT).
Cutthroat trout	*Salmo clarki*	Allison et al., 1964 (DDT, malathion); Andrews et al., 1966 (heptachlor); Eller, 1971 (endrin); Van Valin et al., 1968 (mirex).
Lake trout	*Salvelinus namaycush*	Eller, L. L., unpublished, in Couch, 1975 (aroclor 1248, chlordane); Walsh and Ribelin, 1975 (DDT, dieldrin, endosulfan, 2,4-D, malathion, sevin, atrazine).
Rainbow trout	*Salmo gairdneri*	Abel and Skidmore, 1975 (SLS); Benville et al., 1968 (DMSO); Burkhalter and Kaya, 1977 (NH_3); Chliamovitch and Kuhn, 1977 [bis(tri-*n*-butyltin) oxide]; Christie and Battle, 1963 (TFM); Cope, 1966 (methoxychlor); Gingerich et al., 1978 (carbon tetrachloride); Halver et al., 1962 (DDT); Hawkes, 1977 (whole crude oil); Lowe, 1967 (sevin); Matton and LaHam, 1969 (dylox); McBride et al., 1975; 1979 (kanamycin, landfill leachates); Mitrovic et al., 1968 (phenol); Nestel and Budd, 1975 (aroclor 1254); Racicot et al., 1975 (carbon tetrachloride); Sivarajah et al., 1978 (Aroclor 1254); Smith and Piper, 1972, 1975 (formalin, NH_3); Wedemeyer et al., 1979 (ozone); Wobeser, 1975a,b ($HgCl_2$, CH_3HgCl_2); Wood, E. M., unpublished, in Couch, 1975 (endrin, heptachlor); Wood, E. M., unpublished, in Walsh and Ribelin, 1975 (DDT, lindane, methoxychlor, toxaphene, malathion, methyl parathion).
Steelhead trout	*Salmo gairdneri*	Wedemeyer and Yasutake, 1974, 1978 (formalin, NO_2).
Trout	Not specified	Reichenbach-Klinke, 1975 (bradophen, $CuSO_4$, malachite green, methylene blue, neguvon-masoten, sulfonamides).
		Salmon
Chinook salmon	*Oncorhynchus tshawytscha*	Benville et al., 1968 (DMSO); Buhler et al., 1969 (DDT); DeCew, 1972 (mixture penicillin, streptomycin and oxytetracycline); Hawkes et al., 1980 (miscellaneous PCBs and petroleum hydrocarbons); Wedemeyer and Yasutake, 1974 (formalin); Wood et al., 1957 (sulfamethazine).
Coho salmon	*Oncorhynchus kisutch*	Benville et al., 1968 (DMSO); Buckley et al., 1976 (chlorinated wastewater); Buckley, 1977 (chlorinated wastewater); Hawkes, 1977 (saltwater-soluble crude oil); Hendricks, 1979 (dicamba, esteron, tordon 101, tordon 22k, acrolein, amitrole-T, atrazine, dinoseb, diquat, paraquat-CL); Schneider et al., 1980 (tobramycin); Walsh and Ribelin, 1975 (DDT, dieldrin, endosulfan, 2,4-D, malathion, sevin, atrazine).
Sockeye salmon	*Oncorhynchus nerka*	Benville et al., 1968 (DMSO).

Table 2 Aquatic Species Examined for Pathological Changes in Tissues following Exposures to Organic and Inorganic Environmental Contaminants and Chemotherapeutic Agents (*Continued*)

Common name	Scientific name	Literature source
		Sunfish
Bluegill	*Lepomis macrochirus*	Andrews et al., 1966 (heptachlor); Cope, 1963, 1966 (heptachlor, dichlobenil); Cope et al., 1969, 1970 (dichlobenil, 2,4-D); Eller, L. L., unpublished in Couch, 1975 (abate); Gilderhaus, 1966 ($NaAsO_2$); Kennedy et al., 1970 (methoxychlor); McCraren et al., 1969 (diuron); Van Valin et al., 1968 (mirex); Wood, E. M., unpublished, in Walsh and Ribeline, 1975 (kuron, kurosal).
Green sunfish	*Lepomis cyanellus*	Sorensen, 1976 (Na_2HAsO_4).
Redear sunfish	*Lepomis microlophus*	Eller, 1969 (hydrothol 191).
		Eels
American eel	*Anguilla rostrata*	Ball and Baker, 1969–1970 (DDD); Janicki and Kinter, 1971 (DDT).
Lamprey eel	*Petromyzon marinus*	Christie and Battle, 1963 (TFM).
		Minnows and killifish
Bream	*Abramis brama*	Waluga, 1966a,b (phenol).
Carp	*Cyprinus carpio*	Lakota et al., 1978 (methoxychlor, propoxur); Schulz, 1970, 1971 ($NaTA_2$, toxaphene); Sivarajah et al., 1978 (aroclor 1254); Reichenbach-Klinke, 1975 ($CuSO_4$, dipterex, emtrysidina, hexa-ex, malachite green, methylene blue, neguvon-masoten, sulfonamides, thiabendazole, yomesan).
Fathead minnow	*Pimephales promelas*	Mehrle and Mayer, 1975 (toxaphene).
Goldfish	*Carassius auratus*	Bromage and Fuchs, 1976 (SLS, $ZnSO_4$); Grant and Mehrle, 1970 (endrin); Tafanelli and Summerfelt, 1975 ($CdCl_2$); Van Valin et al., 1968 (mirex).
Mummichog	*Fundulus heteroclitus*	DiMichele and Taylor, 1978 (naphthalene); Eisler and Gardner, 1973 ($CdCl_2$ + $CuCl_2$ + $ZnCl_2$); Gardner and Yevich, 1970 ($CdCl_2$); Gardner and LaRoche, 1973 ($CuCl_2$); Gardner, 1975 (methoxychlor, $CdCl_2$, $HgCl_2$, AgCl); Wasserman and Koepp, 1977 ($HgCl_2$).
Sheepshead minnow	*Cyprinodon variegatus*	Couch et al., 1977 (Kepone); Couch et al., 1979 (trifluralin); Lowe, J. I., personal communication, in Couch, 1975 (dursban).
		Aquarium fish
Cichlids	*Chichlasoma nigrofasciatum*	Ozoh, 1979b ($C_4H_6O_4Pb$).
	Herotilapia multispinosa	Jauch, 1979 (fenthion).
	Tilapia leucosticta	Jauch, 1979 (fenthion).
Guppy	*Poecilia reticulata*	Crandall and Goodnight, 1963 (dowicide G); King, 1962 (DDT); Mount, 1962 (endrin); Stock and Cope, 1969 (TEPA).
Medaka	*Oryzias latipes*	Aoki, 1978 (Cd 109).
Zebra fish	*Brachydanio rerio*	Ozoh, 1979a ($CuNO_3$, $PbNO_3$).

Table 2 Aquatic Species Examined for Pathological Changes in Tissues following Exposures to Organic and Inorganic Environmental Contaminants and Chemotherapeutic Agents (*Continued*)

Common name	Scientific name	Literature source
	Asian fish species	
	Heteropneustes fossilis	Bhatnagar and Shrivastava, 1975 ($CuSO_4$).
	Ophiocephalus punctatus	Mathur, 1975 (dieldrin, lindane).
	Trichogaster fasciatus	Mathur, 1975 (dieldrin, lindane).
	Unspecified	Mathur, 1962 (DDT).
	Catfish	
Asian catfish	*Channa punctatus*	Anees, 1976 (diazinon, dimethoate, methyl parathion); Sastry and Gupta, 1978a,b ($HgCl_2$,$PbNO_3$); Sastry and Sharma, 1978 (endrin).
Channel catfish	*Ictalurus punctatus*	Hinton et al., 1978 (miscellaneous PCBs); Kendall, 1977 (CH_3HgCl_2); Klaunig et al., 1979 (miscellaneous PCBs); Mayer et al., 1978 (toxaphene).
	Marine flatfish	
English sole	*Parophrys vetulus*	Hawkes, 1977 (saltwater-soluble crude oil).
Hogchoker	*Trinectes maculatus*	Trump et al., 1975 ($HgCl_2$).
Sand sole	*Psettichthys melanostictus*	Hawkes, 1980 (saltwater-soluble crude oil).
Starry flounder	*Platichthys stellatus*	Hawkes, 1977 (saltwater-soluble crude oil).
Winter flounder	*Pseudopleuronectes americanus*	Baker, 1969 ($CuSO_4$).
	Miscellaneous marine fish	
Atlantic silverside	*Menidia menidia*	Gardner and LaRoche, 1973 ($CuCl_2$); Gardner, 1975, 1978 (whole, saltwater-insoluble, saltwater-soluble crude oil, waste motor oil).
Cunner	*Tautogolabrus adspersus*	Newman and MacLean, 1974 ($CdCl_2$); Payne et al., 1978 (oil slick).
Lisa	*Mugil auratus*	Establier et al., 1978a ($HgCl_2$,CH_3HgCl_2).
Pacific herring	*Clupea pallasii*	Cameron and Smith, 1980 (whole crude oil).
Pinfish	*Lagodon rhomboides*	Lowe et al., 1971b (mirex).
Robalo	*Dicentrarchus labrax*	Establier et al., 1978c ($HgCl_2$).
Sapo	*Halobatrachus didactylus*	Gutierrez et al., 1978 ($CdCl_2$,$HgCl_2$).
Spot	*Leiostomus xanthurus*	Couch, 1975 (Aroclor 1254, sevin); Hawkins et al., 1980 ($CdCl_2$); Johnson, 1968 (endrin); Lowe, 1964, 1965 (toxaphene, endrin).
	Invertebrates	
American oyster	*Crassostrea virginica*	Lowe et al., 1971a, 1972 (Aroclor 1254, DDT, toxaphene, parathion).
Bent-nosed clam	*Macoma nasuta*	Armstrong and Millemann, 1974 (sevin).
Hard clam	*Mercenaria mercenaria*	Fries and Tripp, 1976 (phenol).
Shrimp (langostino)	*Pendeus kerathurus*	Establier et al., 1978b ($CdCl_2$).

compounds. Most lesions have been extremely nonspecific and merely indicative of toxic insult. The nonspecific microscopic lesions reported in fish and shellfish species after exposures to different chemical compounds (see Table 1 for abbreviations of compounds) are summarized below. These lesions occurred either separately or in combination, depending on the compound used. The descriptions and interpretations provided are mostly in the words of the authors cited, which in some cases are vague or ambiguous. For further information on organ function or anatomic terminology not covered in the glossary, the reader should consult Hoar and Randall (1969) or Morton (1971).

Nonspecific Microscopic Lesions

Gills Epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, telangiectasia, edema with epithelial separation from basement membranes, general necrosis, and/or epithelial desquamation have occurred following exposures to certain *organochlorines*: 2,4-D (Walsh and Ribelin, 1975); DDT (Walsh and Ribelin, 1975); dichlobenil (Cope, 1966); dieldrin (Walsh and Ribelin, 1975); diuron (McCraren et al., 1969); endosulfan (Walsh and Ribelin, 1975); endrin (Eller, 1971); esteron (Hendricks, 1979); heptachlor (Andrews et al., 1966; Wood, E.M., unpublished, in Couch, 1975); methoxychlor (Cope, 1966; Lakota et al., 1978); mirex (Van Valin et al., 1968); Tordon 22K (Hendricks, 1979); toxaphene (Lowe, 1964; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *petroleum compounds*: naphthalene (DiMichele and Taylor, 1978); phenol (Fries and Tripp, 1976; Mitrovic et al., 1968; Reichenbach-Klinke, 1965; Waluga, 1966b); salt-water-soluble crude oil (Hawkes, 1977); *organophosphates*: fenthion (Jauch, 1979); malathion (Walsh and Ribelin, 1975; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); methyl parathion (Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *carbamates*: propoxur (Lakota et al., 1978); sevin (Armstrong and Millemann, 1974; Walsh and Ribelin, 1975); *miscellaneous herbicides*: acrolein (Hendricks, 1979); amitrole-T (Hendricks, 1979); atrazine (Hendricks, 1979; Walsh and Ribelin, 1975); dinoseb (Hendricks, 1979); diquat (Hendricks, 1979); hydrothol 191 (Eller, 1969); $NaTA_2$ (Schulz, 1970); paraquat-CL (Hendricks, 1979); *other miscellaneous compounds*: acid-alkaline pH (Day and Garside, 1976); bis(tri-n-butyltin)oxide (Chliamovitch and Kuhn, 1977); DMSO (Benville et al., 1968); SLS (Abel and Sidmore, 1975); TFM (Christie and Battle, 1963); *nitrogenous compounds*: NH_3 (Burkhalter and Kaya, 1977; Smith and Piper, 1975); NO_2 (Wedemeyer and Yasutake, 1978); ***heavy metal salts***: $CdCl_2$ (Gardner and Yevich, 1970); $CuCl_2 + ZnCl_2 + CdCl_2$ (Eisler and Gardner, 1973); $CuSO_4$ (Baker, 1969; Bhatnagar and Shrivastava, 1975); $HgCl_2$ (Establier et al., 1978c; Wobeser, 1975a); CH_3HgCl_2 (Establier et al., 1978a; Wobeser, 1975a,b); $NaAsO_2$ (Gilderhaus, 1966); and *chemotherapeutic agents*: bradophen (Reichenbach-Klinke, 1975); formalin (Smith and Piper, 1972; Wedemeyer and Yasutake, 1974); malachite green (Reichenbach-Klinke, 1975); ozone (Wedemeyer et al., 1979).

Liver Hepatotoxic lesions of fatty infiltration, nuclear or general hypertrophy of hepatocytes, other degenerative changes in parenchyma (cytoplasmic vacuolation, cellular pleomorphism, deposition of bile or ceroid pigments, hydropic degeneration), loss of hepatic glycogen, coagulative hepatocyte necrosis, sinusoidal and vascular congestion, loss of normal muralial architecture, degeneration or necrosis of biliary epithelium, and perivascular or periportal fibrosis have been reported following exposures to several chemical compounds—for a review of pesticide pathology in fish liver up to 1972 see Couch (1975): *organochlorines*: Aroclor 1248 (Eller, L. L., unpublished, in Couch, 1975); Aroclor 1254 (Couch, 1975; Nestel and Budd, 1975; Sivarajah et al., 1978); carbon tetrachloride (Gingerich et al., 1978; Racicot et al., 1975); chlordane (Eller, L. L., unpublished, in Couch, 1975); 2,4,-D (Cope et al., 1970; Walsh and Ribelin, 1975); DDT (King, 1962; Mathur, 1962; Walsh and Ribelin; 1975);

dichlobenil (Cope et al., 1969); dieldrin (Mathur, 1965, 1975; Walsh and Ribelin, 1975); Dowicide G (Crandall and Goodnight, 1963); endosulfan (Walsh and Ribelin, 1975); endrin (Grant and Mehrle, 1970; Lowe, 1965; Mount, 1962; Sastry and Sharma, 1978; Wood, E. M., unpublished, in Couch, 1975); heptachlor (Andrews et al., 1966; Wood, E. M., unpublished, in Couch, 1975); kuron (Wood, E. M., unpublished, in Walsh and Ribelin, 1975); lindane (Mathur, 1975; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); methoxychlor (Cope, 1966; Lakota et al., 1978; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); tordon 101 and 22k (Hendricks, 1979); toxaphene (Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *petroleum compounds*: crude oil (McCain et al., 1978); naphthalene (DiMichele and Taylor, 1978); *organophosphates*: abate (Eller, L. L., unpublished, in Couch, 1975); diazinon (Anees, 1976); dimethoate (Anees, 1976); dursban (Lowe, J. I. personal communication, in Couch, 1975); dylox (Matton and LaHam, 1969); malathion (Walsh and Ribelin, 1975; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); methyl parathion (Anees, 1976; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *carbamates*: propoxur (Lakota et al., 1978); sevin (Couch, 1975; Walsh and Ribelin, 1975); *miscellaneous herbicides*: acrolein (Hendricks, 1979); amitrole-T (Hendricks, 1979); dinoseb (Hendricks, 1979); diquat (Hendricks, 1979); hydrothol 191 (Eller, 1969); paraquat-CL (Hendricks, 1979); *other miscellaneous compounds*: bis(tri-*n*-butyltin)oxide (Chliamovitch and Kuhn, 1977); DMSO (Benville et al., 1968); TFM (Christie and Battle, 1963); *nitrogenous compounds*: NH_3 (Smith and Piper, 1975); *heavy metal salts*: $CdCl_2$ (Gutierrez et al., 1978; Tafanelli and Summerfelt, 1975); $CuCl_2$ (Gardner and LaRoche, 1973); $CuSO_4$ (Baker, 1969); $PbNO_3$ (Sastry and Gupta, 1978b); $HgCl_2$ (Establier et al., 1978a,c; Gutierrez et al., 1978; Sastry and Gupta, 1978a); CH_3HgCl_2 (Establier et al., 1978a; Kendall, 1977); $NaAsO_2$ (Gilderhaus, 1966); and *chemotherapeutic agents*: $CuSO_4$ (Reichenbach-Klinke, 1975); sulfamethazine (Wood et al., 1957); thiabendazole (Reichenbach-Klinke, 1975).

Liver neoplasia (hepatic cell carcinoma, cholangiocarcinoma) has been reported in rainbow trout following controlled exposures to various toxic compounds, most of which have not been potential contaminants in the aquatic environment (Sinnhuber et al., 1976; Grieco et al., 1978; Hendricks et al., 1980a,b,c; Hendricks et al., 1981). Moreover, certain industrial chemicals, such as PCBs, have been implicated as possible causes of liver neoplasia in populations of Atlantic hagfish (Falkmer et al., 1977), English sole (McCain et al., 1977), and Atlantic tomcod (Smith et al., 1979). At present, adequate experimental evidence on the carcinogenicity of PCBs in fish is not available. Nonetheless, investigations of certain other potential aquatic contaminants have shown that they can induce preneoplastic or neoplastic changes in the liver. Hendricks (unpublished data) found that dietary intake of the chlorinated hydrocarbon DDT produced hepatocellular and cholangiolar (Fig. 13) carcinomas in 21 and 69% of male and female rainbow trout, respectively. Hepatoma in DDT-fed rainbow trout was reported by Halver et al. (1962), while adenomatous changes were reported by Cope et al. (1969) in livers of centrarchid fish exposed to the herbicide dichlobenil and in cutthroat trout exposed to endrin (Eller, 1971).

Kidney Nephrotoxic lesions, including degenerative changes in tubular epithelium (cytoplasmic vacuolation, hydropic degeneration, hyperchromatic nuclei), dilation of tubular lumina, proteinaceous or cellular casts within tubular lumina, tubular necrosis and/or epithelial desquamation, necrosis of interstitial hematopoietic tissues, and excessive development of melanomacrophage centers, are some of the changes observed following exposure to: *organochlorines*: Aroclor 1254 (Nestel and Budd, 1975); DDT (King, 1962; Mathur, 1962); endrin (Mount and Putnicki, 1966; Wood, E. M., unpublished, in Couch, 1975); lindane (Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *petroleum compounds*: naphtha-

lene (DiMichele and Taylor, 1978); phenol (Waluga, 1966a); *organophosphates*: diazinon (Anees, 1976); dimethoate (Anees, 1976); methyl parathion (Anees, 1976; Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *miscellaneous herbicides*: acrolein (Hendricks, 1979); amitrole-T (Hendricks, 1979); dinoseb (Hendricks, 1979); diquat (Hendricks, 1979); paraquat-CL (Hendricks, 1979); *other miscellaneous compounds*: DMSO (Benville et al., 1968); *heavy metal salts*: $CdCl_2$ (Gardner and Yevich, 1970; Gutierrez et al., 1978; Hawkins et al., 1980; Newman and MacLean, 1974; Tafanelli and Summerfelt, 1975); mixture of $CdCl_2$, $CuCl_2$, and $ZnCl_2$ (Eisler and Gardner, 1973); $CuCl_2$ (Gardner and LaRoche, 1973); $CuSO_4$ (Baker, 1969; Bhatnagar and Shrivastava, 1975); $HgCl_2$ (Establier et al., 1978c; Trump et al., 1975); CH_3HgCl_2 (Establier et al., 1978a; Wobeser, 1975b); *chemotherapeutic agents*: $CuSO_4$ (Reichenbach-Klinke, 1975); emtrysidina (Reichenbach-Klinke, 1975); formalin (Smith and Piper, 1975); kanamycin (McBride et al., 1975); malachite green (Reichenbach-Klinke, 1975); sulfamethazine (Wood et al., 1957); sulfonamides (Reichenbach-Klinke, 1975).

Intestine Toxic lesions most commonly reported in the intestine (and occasionally pyloric cecae) include hyperemia; degenerative changes in tips of villi; loss of structural integrity of mucosal folds; degeneration of mucosal epithelium (hypertrophy, vacuolation, hyperchromasia) and/or various smooth muscle layers; necrosis and/or desquamation of mucosal epithelium; cellular debris and excessive mucus in gut lumen; increased numbers of mucous goblet cells; vacuolation or necrosis of submucosa; degenerative changes or necrosis of submucosal vasculature; inflammatory infiltration of submucosa and/or lamina propria. These changes have occurred following exposures to *organochlorines*: miscellaneous PCBs (Hawkes et al., 1980); carbon tetrachloride (Gingerich et al., 1978); 2,4-D (Walsh and Ribelin, 1975); DDT (Janicki and Kinter, 1971; King, 1962; Mathur, 1962; Walsh and Ribelin, 1975); dieldrin (Walsh and Ribelin, 1975); endosulfan (Walsh and Ribelin, 1975); *petroleum compounds*: miscellaneous hydrocarbons and PCBs (Hawkes et al., 1980); naphthalene (DiMichele and Taylor, 1978); phenol (Fries and Tripp, 1976); *organophosphates*: diazinon (Anees, 1976); dimethoate (Anees, 1976); malathion (Walsh and Ribelin, 1975); methyl parathion (Anees, 1976); *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); *nitrogenous compounds*: NH_3 (Smith and Piper, 1975); *heavy metal salts*: $CdCl_2$ (Gardner and Yevich, 1970; Gutierrez et al., 1978; Newman and MacLean, 1974); $HgCl_2$ (Establier et al., 1978a,c; Gutierrez et al., 1978; Sastry and Gupta, 1978a); CH_3HgCl_2 (Establier et al., 1978a); $PbNO_3$ (Sastry and Gupta, 1978b).

Spleen Atrophy, mottled or blanched in color, hypocellularity (reduction in red and white pulp), fibroplasia, and subcapsular necrosis of blood cells have been observed with *organochlorines*: Aroclor 1254 (Nestel and Budd, 1975); carbon tetrachloride (Gingerich et al., 1978); 2,4-D (Walsh and Ribelin, 1975); DDT (King, 1962; Walsh and Ribelin, 1975); dieldrin (Walsh and Ribelin, 1975); endosulfan (Walsh and Ribelin, 1975); *organophosphates*: malathion (Walsh and Ribelin, 1975); *carbamates*: sevin (Walsh and Ribelin, 1975); *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); *other miscellaneous compounds*: DMSO (Benville et al., 1968); *nitrogenous compounds*: NH_3 (Smith and Piper, 1975); and *chemotherapeutic agents*: formalin (Smith and Piper, 1972).

Pancreas Islet cell hyperplasia, subcapsular and acinar cell necrosis, vascular congestion, infarction, and edema have been reported following exposures to *organochlorines*: Dowpon (Schulz, 1971); endrin (Eller, 1971); *petroleum compounds*: naphthalene (DiMichele and Taylor, 1978); *organophosphates*: abate (Eller, L. L., unpublished, in Couch, 1975); *other miscellaneous compounds*: DMSO (Benville et al., 1968); and *chemotherapeutic agents*: sulfamethazine (Wood et al., 1957).

Adrenal Cortex Interrenal cell necrosis, cellular and nucleolar hyperplasia and hypertrophy,

nuclear hypertrophy, hyperchromatic cytoplasm, cytoplasmic vacuolation, atrophy of cells with clumping of nucleoplasm, high mitotic index, and prominent sinusoids have been observed after exposures to *organochlorines*: DDT (King, 1962); dieldrin (Walsh and Ribelin, 1975); *petroleum compounds*: naphthalene (DiMichele and Taylor, 1978); *other miscellaneous compounds*: SLS (Bromage and Fuchs, 1976); *nitrogenous compounds*: landfill leachates (McBride et al., 1979); *heavy metal salts*: $ZnSO_4$ (Bromage and Fuchs, 1976); and *chemotherapeutic agents*: formalin (Smith and Piper, 1972).

Thyroid Gland Reduced follicular height and possible hyperplasia have been observed with *organochlorines*: endrin (Grant and Mehrle, 1970) and *heavy metal salts*: $CdCl_2$ (Gardner, 1975).

Hepatopancreas (digestive diverticula in some invertebrates) Atrophy, reduction in height of tubular epithelium, tubular dilation, necrosis, and desquamation of tubular epithelium have been noted after exposures to *organochlorines*: Aroclor 1254 (Lowe et al., 1972); DDT (Lowe et al., 1971a; toxaphene (Lowe et al., 1971a); *petroleum compounds*: phenol (Fries and Tripp, 1976); *organophosphates*: methyl parathion (Lowe et al., 1971a); and *heavy metal salts*: $CdCl_2$ (Establier et al., 1978b).

Testes Stimulation of spermatogenesis and exhaustion atrophy, development of ova-like cells within follicles, general atrophy and hypospermia (lower mean index of spermatogenic development), necrosis of tubular boundary cells with hemorrhage, vasodilation, and congestion, increased numbers of infiltrating macrophages with phagocytized debris, necrosis of primary germ cells with atrophy of seminiferous tubules, fibrosis, and infiltration of mononuclear inflammatory cells have been observed with *organochlorines*: kuron (Wood, E. M., unpublished, in Walsh and Ribelin, 1975); *miscellaneous herbicides*: hydrothol 191 (Eller, 1969); *other miscellaneous compounds*: TEPA (Stock and Cope, 1969); and *heavy metal salts*: $CdCl_2$ (Sangalang and O'Halloran, 1972; Tafanelli and Summerfelt, 1975).

Ovaries Hyperplasia of germinal epithelium and involution of some ova, decreased frequency of oocyte maturation, cytoplasmic clumping, and fragmentation and karyolysis of ova have been observed after exposure to *organochlorines*: Aroclor 1254 (Sivarajah et al., 1978) and endrin (Eller, 1971); and *heavy metal salts*: $CdCl_2$ (Tafanelli and Summerfelt, 1975) and $NaAsO_2$ (Gilderhaus, 1966).

Stomach Tunica muscularis with nuclear depolarization and cytoplasmic hyperchromasia, general edema, loss of pepsinogen granules from chief cells, disintegration of goblet cells, and degeneration and pyknosis of glandular epithelium with desquamation of gastric mucosa have occurred following exposures to *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); *heavy metal salts*: $CuSO_4$ (Bhatnagar and Shrivastava, 1975); $HgCl_2$ (Sastry and Gupta, 1978a); CH_3HgCl_2 (Establier et al., 1978a); and *chemotherapeutic agents*: sulfamethazine (Wood et al., 1957).

Esophagus Vacuolation and nuclear condensation (pyknosis) of mucosal epithelial cells and necrosis of all four tunics have been observed with *miscellaneous herbicides*: acrolein (Hendricks, 1979) and *other miscellaneous factors*: acid-alkaline pH (Daye and Garside, 1976).

Rectum Erosion of mucosa at tips of the villi have been reported after exposure to the *heavy metal salt*: $HgCl_2$ (Sastry and Gupta, 1978a).

Heart Degeneration of ventricular myocardium, hypotonicity with poor circulation, and hemostasis have been observed with *petroleum compounds*: whole crude oil (Gardner, 1975) and *heavy metal salts*: $C_4H_6O_4Pb$ (Ozoh, 1978b).

Blood Vessels Vascular anomalies, periodic acid-Schiff (PAS)-positive globular masses in lumina, endothelial separation from basement membrane, subendothelial myositis, and necrosis of arterial walls have been reported after exposures to *organochlorines*: 2,4-D (Cope et al., 1970); *petroleum compounds*: waste motor oil (Gardner, 1978); *miscellaneous herbicides*: hydrothol 191 (Eller, 1969); *heavy metal salts*: $NaAsO_2$ (Gilder-

haus, 1966); and *chemotherapeutic agents*: sulfamethazine (Wood et al., 1957).

Peripheral Blood Microcytic, hypochromic, hemolytic anemia with high percentages of abnormal and immature erythrocytes; formation of Heinze and Howell-Jolly bodies; poikilocytosis, anisocytosis, hyperchromasia, anisochromasia, and presence of "smudge" cells among erythrocytes; polycythemia; morphological alterations of erythrocyte nuclei including karyorrhexis; neutrophilia; eosinophilia with deformed nuclei, cytoplasmic vacuolation, and reduction in granular mass; and irregularly shaped thrombocyte nuclei have been observed with *organochlorines*: heptachlor (Andrews et al., 1966); chlorinated wastewater (Buckley et al., 1976; Buckley, 1977); *heavy metal salts*: $CdCl_2$ (Gardner and Yevich, 1970; Gardner, 1975; Gutierrez et al., 1978; Newman and MacLean, 1974); and *chemotherapeutic agents*: formalin (Smith and Piper, 1972); methylene blue (Reichenbach-Klinke, 1975); ozone (Wedemeyer et al., 1979).

Skeletal Muscle Edema of myotomes, degenerative myotomal atrophy, and necrosis have been observed with *petroleum compounds*: naphthalene (DiMichele and Taylor, 1978); *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); $NaTA_2$ (Schulz, 1970); and *other miscellaneous compounds*: TFM (Christie and Battle, 1963).

Brain Hyperemia, hemorrhage, vascular congestion and dilation, infarction, cerebral edema, nuclear pyknosis, rupture and hemorrhage of meninx primitiva, and swelling of myelin sheaths around nerve fibers have occurred with exposures to *organochlorines*: 2,4-D (Cope et al., 1970; Walsh and Ribelin, 1975); DDT (Walsh and Ribelin, 1975); dieldrin (Walsh and Ribelin, 1975); endosulfan (Walsh and Ribelin, 1975); methoxychlor (Kennedy et al., 1970); *petroleum compounds*: naphthalene (DiMichele and Taylor, 1978); *organophosphates*: malathion (Walsh and Ribelin, 1975); *other miscellaneous compounds*: DMSO (Benville et al., 1968); *heavy metals*: $^{109}Cd^{+2}$ (Aoki, 1978); $CuCl_2$ (Gardner and LaRoche, 1973); and *chemotherapeutic agents*: neguvon and masoten (Reichenbach-Klinke, 1975).

Spinal Cord Swelling of lipoid substance surrounding large nerve cells and "spirality" (twisting of the cord) have been observed with *heavy metal salts*: $CuNO_3$ (Ozoh, 1979a) and *chemotherapeutic agents*: dipterex (Reichenbach-Klinke, 1975).

Integument Reduction in mucous cell numbers and mucous secretion; increased thickness of epidermis; hypertrophy of squamous cells and mucous goblet cells; hyperplasia of mucous goblet cells; hypersecretion of mucus; abnormal melanogenesis of melanophores; generalized inflammation; hemorrhage, necrosis, and erosion of epithelium in pharynx, fins, and tail; epithelial vacuolation, and pyknosis with desquamation have been observed with *organochlorines*: toxaphene (Mayer et al., 1978); *petroleum compounds*: salt-water-soluble crude oil (Hawkes, 1977); phenol (Mitrovic et al., 1968; Reichenbach-Klinke, 1965; Waluga, 1966a); *organophosphates*: malathion (Walsh and Ribelin, 1975); *carbamates*: sevin (Armstrong and Millemann, 1974); *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); *other miscellaneous compounds*: bis(tri-*n*-butyltin) oxide (Chliamovitch and Kuhn, 1977); acid-alkaline pH (Daye and Garside, 1976); *heavy metal salts*: $CdCl_2$ (Newman and MacLean, 1974); $CuCl_2 + CdCl_2 + ZnCl_2$ (Eisler and Gardner, 1973); $C_4H_6O_4Pb$ (Ozoh, 1979b); $PbNO_3$ (Ozoh, 1979a); and *chemotherapeutic agents*: emtrysidina (Reichenbach-Klinke, 1975); hexa-ex (Reichenbach-Klinke, 1975); malachite green (Reichenbach-Klinke, 1975).

Lateral Line Organs Necrosis of sensory and sustentacular epithelium with pyknosis and karyorrhexis; necrosis of cuboidal cells lining canal; infiltration of granular leukocytes in canal lumina, walls, and peripheral connective tissues; reduction of goblet cell numbers lining canal; neuromast nuclei with enlarged nucleoli; and loss of nuclei in some cells have been reported following exposures to *organochlorines*: methoxychlor (Gardner, 1975); *petroleum compounds*: naphtha-

lene (DiMichele and Taylor, 1978); and *heavy metal salts*: $CuCl_2$ (Gardner and LaRoche, 1973); $CuCl_2 + ZnCl_2 + CdCl_2$ (Eisler and Gardner, 1973); $HgCl_2$ (Gardner, 1975); CH_3HgCl_2 (Gardner, 1975); AgCl (Gardner, 1975).

Olfactory Organs Hyperplasia and cytoplasmic degeneration of neurosensory and sustentacular epithelium with cystlike formations containing cellular debris and remnants of sensory tissue; necrosis of mucosa with cellular debris in lumina, vasodilation, and congestion in submucosa, vacuolation and pyknosis in cells of lamina propria; epithelial metaplasia with replacement of neurosensory and sustentacular cells by a less differentiated epithelium and appearance of extracellular refractile rods in mucosa; and hypertrophy and hyperplasia of mucous goblet cells in mucosal lining have been observed with *petroleum compounds*: whole, saltwater-insoluble, and saltwater-soluble crude oil (Gardner, 1975); naphthalene (DiMichele and Taylor, 1978); *other miscellaneous factors*: acid-alkaline pH (Daye and Garside, 1976); and *heavy metal salts*: $CuCl_2$ (Gardner and LaRoche, 1973); $HgCl_2$ (Gardner, 1975); AgCl (Gardner, 1975).

Eye Enlarged abnormally soft lens; degeneration of lens fibers; other degenerative changes such that boundaries between capsule, lens epithelium, and fibers become indistinct; enlargement of lens capsule; corneal degeneration with necrosis of external squamous epithelium and vacuolation of basal epithelium; desquamation of corneal epithelium preceded by poor differentiation between epithelium and substantia propria; swelling of substantia propria after loss of corneal epithelium; hemorrhage in anterior chamber; hemorrhage in periorbital connective tissues; hemorrhage and leukocyte infiltration of limbus corneae; anophthalmia, microphthalmia, and exophthalmia have occurred after exposures to *petroleum compounds*: whole crude oil (Hawkes, 1977); oil slick (Payne et al., 1978); *carbamates*: sevin (Walsh and Ribelin, 1975); *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975); *other miscellaneous compounds*: bis(tri-*n*-butyltin) oxide (Chliamovitch and Kuhn, 1977); acid-alkaline pH (Daye and Garside, 1976); and *heavy metal salts*: $CuCl_2$ (Gardner and LaRoche, 1973); $C_4H_6O_4Pb$ (Ozoh, 1979b).

Pseudobranch Degeneration of secretory cells and hypertrophy of epithelial cells with nuclear swelling and occasional ballooning degeneration have been observed with *petroleum compounds*: whole crude oil (Gardner, 1975); *heavy metal salts*: CH_3HgCl_2 (Wobeser, 1975b); and *chemotherapeutic agents*: formalin (Smith and Piper, 1972).

Yolk Sac Developmental retardation with failure to absorb the yolk sac and occurrence of blue sac disease syndrome have been reported following exposures to *nitrogenous compounds*: NH_3 (Burkhalter and Kaya, 1977) and *heavy metal salts*: $PbNO_3$ (Ozoh, 1979a).

Swim Bladder Air distension in fry and edema have been reported after exposures to *organochlorines*: DDT (Burdick et al., 1964) and *miscellaneous herbicides*: atrazine (Walsh and Ribelin, 1975).

Pituitary Gland Degranulation of cells has been reported following exposure to the *organochlorine* DDD (Ball and Baker, 1969–1970).

Embryonic Tissues Teratomas have occurred in up to 15% of the progeny from parent fish exposed to a mixture of *chemotherapeutic agents*: penicillin G procain, dihydrostreptomycin sulfate, oxytetracycline (DeCew, 1972).

Microscopic Lesions of Diagnostic Significance

Some lesions that occur in fish appear to be useful in diagnosing exposures to certain toxic chemicals once nontoxic causes have been ruled out. Carbaryl (sevin) poisoning produces a syndrome characterized by hemorrhages within white skeletal muscle adjacent to the vertebral column, atrophy of the red skeletal muscle parallel to the lateral line, myxomatous degeneration of fat, and vacuoles in the molecular layer of the optic tectum and in the lateral geniculate body of the brain (Walsh and Ribelin, 1975).

Vertebral deformities are less specific but are suggestive of poisoning by several toxic compounds. For example, Kepone exposure of sheepshead minnows causes a neurologic dysfunction that is clinically observable as tetany or chronic rigor in skeletal muscle (Couch et al., 1977). This results in loss of control of caudal melanocyte patterns (blacktail), myotomal distortion, scoliosis, and fractures of vertebral centra in severe cases. These fractures undergo osteoblastic repair but, depending on severity, can cause further neurologic damage by compression of the lateral funiculi of the spinal cord. Toxaphene poisoning in fish appears similar to Kepone poisoning in that vertebral deformities occur; however, the mechanism of lesion production is different. Collagen metabolism is altered since toxaphene causes a functional vitamin C deficiency, resulting in reduced vertebral collagen and hence vertebral hyperfragility (Mehrle and Mayer, 1975; Mayer et al., 1978). Trifluralin exposure of sheepshead minnows produces a vertebral lesion that is unique in the literature of fish pathology (Couch et al., 1979). The resulting syndrome is one of hyperostosis (bone hypertrophy), characterized by vertebral dysplasia (Figs. 5 and 6, pages 287 and 288) seen as nearly symmetrical hypertrophy of vertebrae with foci of osteoblasts and fibroblasts actively producing bone and bone precursors. Hypertrophy of the notochord, compression of the spinal cord and mesonephric ducts of the kidney from vertebral outgrowths, and fusion of the vertebrae are accompanying lesions. Mechanisms proposed for trifluralin poisoning include alteration of calcium metabolism in the corpuscles of Stannius and the ultimobranchial glands, affecting bone deposition and/or direct or indirect stimulation of osteogenic cells. Additional compounds reported to produce vertebral deformities (scoliosis, lordoscoliosis) include malathion (Mount and Stephen, 1967; Weis and Weis, 1976), other organophosphates (akton, methyl parathion, trichlorfon, phosalone, demeton) (McCann and Jasper, 1972), carbamates (sevin) (Carter, 1971), and lead salts (Holcombe et al., 1976; Ozoh, 1979b).

In both humans (Robbins, 1974) and animals (Smith et al., 1972), bloodborne toxic chemicals do not usually cause glomerular lesions; instead, they produce tubular nephroses. As a rule, chemicals toxic to liver parenchyma often affect tubular epithelium to some degree (Smith et al., 1972). Soluble salts of mercury are noted for extreme nephrotoxicity and certain fish species are susceptible as well [hogchoker, *Trinectes maculatus* (Trump et al., 1975); mummichog (Wassermann and Koepp, 1977)]. Wobeser (1975b) and Establier et al. (1978a,c) noted that mercury and methyl mercury exposure of three fish species—rainbow trout, lisa (*Mugil auratus*), and robalo (*Dicentrarchus labrax*)—resulted in both tubular and glomerular lesions. This does not mean that toxic glomerular nephrosis in fish is a specific lesion for mercury poisoning, but neither is it common. Therefore, it may prove useful in narrowing the choice of a causative compound in a diagnostic situation. Other toxic chemicals that also produce some degree of glomerular change as well as other lesions in fish are cadmium (Hawkins et al., 1980), DMSO (Benville et al., 1968), erucic acid in rapeseed oil (Hendricks, J. D., unpublished data), pyrrolizidine alkaloids in tansy ragwort (Hendricks et al., 1981), amitrole-T (Fig. 12) (Hendricks, 1979), endrin (Mount and Putnicki, 1966), and methyl parathion (Wood, E. M., unpublished, in Walsh and Ribelin, 1975).

It should be noted in this section that the PAS-positive globules found within the vasculature of bluegills exposed to 2,4-D (Cope et al., 1970) are not considered a specific lesion. These globules have also been reported in bluegills exposed to methoxychlor (Kennedy et al., 1970) and in redear sunfish treated with hydrothol 191 (Eller, 1969). They are considered to be a nonspecific response to toxicants peculiar to centrarchid species (Walsh and Ribelin, 1975).

Ultrastructural Pathology in Aquatic Toxicology

Neoplasia is a pathological event which undoubtedly involves a series of complex ultra-

structural preneoplastic changes, most of which cannot be recognized by light microscopy until overt cell transformation and tumor growth has occurred. Most of these changes and their chronology of occurrence are still to be defined by TEM and SEM. Since many of the pollutants in the aquatic environment are potential carcinogens, ultrastructural research should prove to be valuable for monitoring neoplasia in wild populations of aquatic species chronically exposed to low levels of various toxic compounds. Investigation of ultrastructural changes other than those of neoplasia is equally important for gaining insight into the environmental impact of various toxicants. The groups of compounds that have been studied most include organochlorines, petroleum compounds, and heavy metal salts. Following is a summary of ultrastructural lesions that have occurred in fish tissues after exposure to these and other groups of compounds. As with most toxicological lesions observed with light microscopy, the ultrastructural pathologies appear to be nonspecific with respect to the causative toxicant.

Gills

Chloride cell degeneration with hypertrophied perinuclear spaces and smooth endoplasmic reticulum; mitochondria having disorganized cristae and ruptured membranes; formation of autophagosomes, vacuoles, myelinlike bodies, and apical vesicles in epithelial cytoplasm; and reduction in thickness of apical homogeneous epithelial cytoplasm have been reported with *petroleum compounds*: saltwater-soluble crude oil (Hawkes, 1977); *other miscellaneous compounds*: bis(tri-*n*-butyltin) oxide (Chliamovitch and Kuhn, 1977); and *heavy metal salts*: $CuSO_4$ (Baker, 1969).

Liver

Distinct vacuolation of hepatocytes, enlargement and/or vesiculation of rough endoplasmic reticulum, presence of circular arrays of smooth-surfaced membranes and myelinlike bodies in hepatocyte cytoplasm, proliferation and "bizarre" whorls of smooth and rough endoplasmic reticulum, loss of glycogen reserves in hepatocytes and presence of cochlear ribosomes, presence of intranuclear and intracytoplasmic electron-dense particles, increased size and numbers of lipofuscin granules, and abnormally enlarged mitochondria have been reported with *organochlorines*: Aroclor 1254 (Sivarajah et al., 1978); miscellaneous PCBs (Hawkes, 1980; Hinton et al., 1978; Klaunig et al., 1979); *petroleum compounds*: whole crude oil (Hawkes, 1977); and *heavy metal salts*: Na_2HAsO_4 (Sorensen, 1976).

Kidney

Focal degeneration of first and second proximal tubules with granular, vacuolated cytoplasm containing swollen or dense contracted mitochondria, some having a granular matrix and focal electron densities, epithelium of third proximal tubules having increased numbers of vacuoles, formation of autophagosomes, lipid droplets, nuclei with marginated chromatin, swollen nuclear envelopes and basal membranes contorted into myelinlike figures, distortion of tubular microvilli, and dilation of endoplasmic reticulum have been reported with *heavy metal salts*: $CdCl_2$ (Hawkins et al., 1980) and $HgCl_2$ (Trump et al., 1975; Wasserman and Koepp, 1977).

Intestine

Absence or reduction of brush border in mucosal epithelium, presence of abnormal inclusion bodies throughout cytoplasm or in apical cytoplasm of columnar epithelium, cytoplasmic vesiculation in columnar cells near luminal surface, abnormal reduction in cytoplasm density, increased amounts of rough endoplasmic reticulum in basal mucosal cells, and diffuse distribution of mitochondria have been reported with *organochlorines*: miscellaneous PCBs (Hawkes, 1980) and *petroleum compounds*: miscellaneous hydrocarbons (Hawkes, 1980).

Pancreas

Submicroscopic degeneration of acinar cells of exocrine pancreas has been reported following

exposure to the *organochlorine* Dowpon (Schulz, 1971).

Testes

Damage to head region of spermatozoa and loss of serrations in outer membrane have been reported with the *organochlorine* Aroclor 1254 (Sivarajah et al., 1978).

Ovaries

Enlargement and proliferation of smooth endoplasmic reticulum have been observed with the *organochlorine* Aroclor 1254 (Sivarajah et al., 1978).

Skeletal Muscle

Numerous swollen mitochondria with some disruption of internal membranes and cristae and intercellular breakdown of membranes have been observed with *petroleum compounds*: whole crude oil (Cameron and Smith, 1980).

Brain

Enlarged and irregularly shaped non-membrane-bound perinuclear and intracellular spaces have been observed following exposure to *petroleum compounds*: whole crude oil (Cameron and Smith, 1980).

Integument

Increased numbers of mucus glands appearing dilated have been observed (with SEM) after exposure to *petroleum compounds*: saltwater-soluble crude oil (Hawkes, 1977).

Neurosensory Epithelium

Severe damage to receptor organelles in nares epithelium, including degeneration of chemosensory cilia and loss of microridges circumscribing epithelial cells surrounding olfactory organs, has been observed (with SEM) following exposure to *petroleum compounds*; saltwater-soluble crude oil (Hawkes, 1980).

BIOLOGICAL SIGNIFICANCE OF TOXIC PATHOLOGY

When an aquatic species encounters a toxic compound, the effect is direct, induced, or indirect. A direct effect is caused by direct action of the toxic substance on the aquatic organism. The most obvious direct effect is acute and consists of irreversible damage to vital organ function, resulting in rapid morbidity and death. Most commonly, this occurs as a toxic effect whereby the compound interferes with the integrity of tissue cells or cell processes, and is often manifested by overt gross, histological, and/or ultrastructural lesions. Acute toxic effects are often recognized in short exposure periods (hours or days), while longer periods of low-level exposure (weeks or months) may be required to elicit certain chronic effects. Acute direct effects are typical of experimental situations in the laboratory; if they occur in nature the resulting mass mortalities in aquatic animal populations are more likely to be observed than in the other examples discussed below.

A chronic direct effect differs from an acute one in that the toxicant causes a sublethal change in the host which may or may not be the eventual cause of death. Sublethal changes can occur from a single encounter or from continuous exposure to a toxicant over a long period of time. If a sublethal change is debilitative and predisposes the host to other environmental forces (infectious disease, predation), then the ensuing process (disease or predation) is said to be "induced." Of course, this can occur only if the environmental forces are present. Therefore, an induced effect is brought about by direct action of the toxicant on the aquatic organism but can occur only in the presence of another factor or agent. Chronic direct effects and induced effects of toxicant exposure are of particular importance to those concerned with the aquatic environment since they more closely represent natural situations: toxicants purposely or accidentally released into an aquatic environment are diluted rapidly by ambient waters to levels that are usually marginally lethal and very

often sublethal. Exceptions do occur, with large volumes of toxic materials released in short periods of time, particularly at point sources of industrial effluents or ocean acid dump sites. In these cases, toxicant levels may be lethal and aquatic organisms in the immediate vicinity may suffer acute direct effects resulting in mortality. Actual animal losses occurring within a wild population due to chronic direct or induced effects have not been documented because it is nearly impossible to corroborate such data. This is because affected animals are eliminated randomly from the population by natural means which mask the real causative factors. For example, experimental and circumstantial evidence has shown that certain toxic compounds can disrupt sensory perceptions by affecting the lateral line and olfactory organs (Eisler and Gardner, 1973; Gardner and LaRoche, 1973; Gardner, 1975; Daye and Garside, 1976; DiMichele and Taylor, 1978; Hawkes, 1980) or the functioning of the eyes (Walsh and Ribelin, 1975; Daye and Garside, 1976; Chliamovitch and Kuhn, 1977; Hawkes, 1977; Payne et al., 1978; Ozoh, 1979b). These pathologies are chronic direct effects that would certainly adversely affect vital behavior patterns such as schooling, migration, prey selection or capture, and predator escapement, making survival unlikely.

That certain chemical compounds are carcinogenic to various fish species has been experimentally established. Neoplasia is a potentially life-threatening disease for most hosts and could be categorized as an acute direct effect, as discussed earlier. However, in the case of aquatic invertebrates and lower vertebrates, the chemically induced neoplasia reported in most studies may be more correctly classified as a chronic direct effect. Many such tumors are benign, involving nonvital tissues, and those that are malignant enlarge and metastasize so slowly that the life of the host is not immediately threatened. We have observed for nearly 16 mo a group of 100 rainbow trout exposed as embryos to the carcinogen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine. Nearly all of these fish developed nephroblastomas and hepatic cellular carcinomas, which were grossly visible as exaggerated bulges and swellings protruding from the abdominal area. Although a few mortalities (nine) have occurred, most of the affected fish are alive and feeding at this writing. Quite remarkably, this survival is despite severe debilitation of kidney and liver as well as other organ functions due to the massive displacement of normal tissues by the neoplastic growths. These tumors will eventually kill all of the fish in the tank. However, if the fish had been removed from the near-optimum conditions of the laboratory to a natural environment, they would have died early after tumor formation due to the selective processes of predation, secondary diseases, intra- and interspecific competition, and social hierarchy. Exceptions have been reported in which neoplasia appeared to be an acute direct effect causing mass mortalities: a captive population of feral kelp bass sustained mortalities from metastatic thyroid adenocarcinomas thought to have been caused by water quality factors (Blasiola et al., 1981); mortalities in certain bivalve mollusk populations exposed to possible pollutants have been associated with malignant neoplasms (Christensen et al., 1974; Mix et al., 1977b; Brown et al., 1977). For further information on neoplasia in lower vertebrates and invertebrates, the investigator is referred to the yearly activities report of the Registry of Tumors in Lower Animals, Smithsonian Institution (1979).

Physiological stress accompanies toxicant insult, and it has been shown to exert a chronic direct effect by adversely altering endocrine functions, as sometimes manifested by changes in endocrine histology and histochemistry. Abnormally high blood cortisol levels have been reported in goldfish exposed to sodium lauryl sulfate (Bromage and Fuchs, 1976), killifish exposed to naphthalene (DiMichele and Taylor, 1978), and steelhead trout exposed to therapeutic formalin (Wedemeyer and Yasutake, 1974). Cortisol, a glucocorticoid hormone, plays a major regulatory role in normal metabolism and thus influences the behavior of an individual (Prosser, 1973). In

addition, production of androgenic hormones, which are essential for development of male accessory and secondary sexual characteristics and sexual behavior (Prosser, 1973), has been shown to be reduced in testes of brook trout exposed to cadmium chloride (Sangalang and O'Halloran, 1972). For survival of an individual or a population of organisms, normal behavior patterns are as essential as normal functioning of physiological processes and vital organs. Aberrant behavior invites predation or starvation and, when associated with the reproductive cycle, assures reduced population recruitment.

Toxicant-related physiological stress may also lead to induced effects. The physiological stress can directly enhance a host's susceptibility to infectious agents (latent viruses, bacteria, protozoans, etc.) endemic in a population of aquatic animals. The resulting induced effect is clinical disease that would not have surfaced in an otherwise healthy population of animals. Suppression of immunological competence and thus host resistance may be an important factor. The following are examples of the toxicant-infectious disease synergism: penaeid shrimp exposed to low levels of Aroclor 1254 showed a higher prevalance and intensity of infection by a shrimp-specific baculovirus (Couch and Courtney, 1977); rainbow trout exposed to copper showed greater susceptibility to infection with IHN virus (Hetrick et al., 1979); goldfish exposed to mirex developed clinical mycobacterial infections (Van Valin et al., 1968); mullet exposed to crude oils experienced outbreaks of bacterial fin rot disease (Giles et al., 1978); and American oysters exposed to a mixture of DDT, toxaphene, and parathion developed a secondary infection with a mycelial fungus (Lowe et al., 1971a). Surface lesions, resulting from contact with a toxicant, provide direct portals of entry for secondary bacterial or fungal infections, which can ultimately lead to death of the host. Pollutant-related infectious diseases are discussed by Brown et al. (1977).

Indirect effects represent another complex relationship, resulting from the action of a toxicant on something other than the aquatic organism which in turn has an effect on that organism. This can be illustrated by reviewing the use of the larvicide TFM to control the parasitic lamprey eel (*Petromyzon marinus*) in the Great Lakes commercial fishery during the early 1960s. TFM, applied in low doses, selectively killed lamprey larvae but left desirable fish species unharmed (Applegate and King, 1962). The indirect effect was a decline in fish losses due to lampreys and subsequent increase in commercial fish yield. However, as one might surmise, most toxicant-related indirect effects are deleterious rather than beneficial. A toxicant may selectively kill forage species of organisms, such as plankton, insects, or bait fish, which may be more susceptible to its lethal properties. Organisms that depend on those species as a food source are indirectly affected by having to find other forage or starve. Toxicants may exert additional indirect effects by adversely altering the physical properties of the aquatic environment, making survival for organisms difficult or impossible; for instance, the use of herbicidal compounds, such as copper sulfate, may result in anoxic (lack of dissolved oxygen) conditions caused by large quantities of dead and decaying aquatic vegetation (Surber and Everhart, 1950).

In addition to toxicants proved to be carcinogenic, there are many others that are suspected of being carcinogenic, perhaps because their residues have been detected in the tissues of neoplastic animals or at least within the ambient water or bottom sediments. As already noted, PCBs have been implicated in several examples of spontaneous neoplasia in feral finfish populations. Petroleum hydrocarbons have also been suspected as carcinogenic by those reporting neoplasia in bivalve mollusk species exposed to oil spills (Barry and Yevich, 1975; Brown et al., 1977; Yevich and Barszcz, 1977; Mix et al., 1977a,b, 1979; Lowe and Moore, 1978; Harshbarger et al., 1979). Unfortunately, there is little experimental evidence showing whether these two groups of compounds or certain others are indeed carcinogenic within a defined set of circumstances. To

pursue these studies further, aquatic toxicologists will have to address the following problems: (1) difficulties in experimentally duplicating the environmental variables that exist in nature, particularly synergistic effects of behavioral, physical, and chemical parameters that may contribute to carcinogenesis; (2) technical inability to maintain certain aquatic fish and shellfish species in the laboratory; (3) lack of well-defined sensitive aquatic animal models for screening and investigation of the neoplastic process; and (4) inability or failure to maintain controlled sublethal toxicant exposures for periods of time long enough (12–24 mo) for carcinogenesis to occur.

SUMMARY

The far-reaching pathological effects of chemical pollution are often very difficult to define in populations of aquatic species. This is particularly true of subtle changes that occur over long periods of exposure to intermittent or continuous sublethal doses of one or several toxicants. It is these changes that aquatic toxicologists and pathologists will be most concerned with in years to come.

The many disciplines in the science of aquatic toxicology are joined in the task of identifying and comprehending each toxicant's mode of activity in organisms which is responsible for clinical signs of intoxication or death. This can be discerned to some degree through biochemical and physiological studies. However, effects are often due to physical changes in the tissues at the cellular or ultrastructural levels and can only be speculated upon unless they are visualized. Since these changes are not grossly apparent, histopathological studies with light microscopy, TEM, and SEM are necessary for the description and evaluation of potential lesions in aquatic animals exposed to various toxicants. Although many toxicant-induced tissue pathologies so far examined have been nonspecific, this is not surprising since aquatic toxopathology is in its infancy. As the science matures and more descriptive studies are made, lesions should emerge which will allow greater specificity in diagnosing exposure to certain groups of compounds or perhaps to single toxicants. By combining histopathological results with results of biochemical and physiological studies, the complete reaction of an aquatic organism to a toxicant may be defined for future diagnostic purposes.

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Editor's note: Two new atlases, on rainbow trout and striped bass, have been published since this chapter was written: Groman DB: Histology of the striped bass. Amer Fish Soc Monogr 3:1–116, 1982; Yasutake WT, Wales JH: Microscopic anatomy of salmonids: An atlas. U.S. Fish Wildl Serv Res Pub 150:1–190, 1983.

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